日本熱帯医学会雑誌

Japanese Journal of Tropical Medicine and Hygiene

第 5 巻　第3,4号　　昭和52年12月15日

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日熱医会誌
Japan. J.T.M.H.

日本熱帯医学会

Japan. J. Trop. Med. Hyg., Vol. 5, No. 3, 4, 1977, pp. 171-181

STUDIES ON THE MORBIDITY OF FOREIGN VISITORS TO A CERTAIN COMPANY IN JAPAN

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Received for publication 11 October 1977

Abstract: Recent papers suggested that as the result of the increase of Japanese travellers abroad, number of patients affected with imported tropical disease increased every year. This fact also implies that foreign visitors are bringing tropical disease into Japan. Therefore, we have conducted a survey of foreign visitors who visited Matsushita Electric Co. Ltd. for the purpose of technical training for short periods from 1972 to 1976, studying the possibility of invasion of tropical diseases by interview and on medical records. The results obtained were as follows:

1. Of total 532 visitors, 485 were from tropical regions and 47 from other countries, and the morbidity rates indicated 20.2 per cent and 29.8 per cent respectively in the observation period of one month following their arrival.
2. Fortunately no tropical diseases such as malaria, filariasis, dengue fever and cholera were found so far.
3. Two cases, a Malaysian with *Tenia saginata* and an Iranian with orchitis following mumps which had affected him for some time before their arrival, were cured.
4. Many visitors from tropical regions were easily affected with common cold within a few days after their arrival, especially in the fall and winter season, as compared with those from Europe and North America.
5. A thorough check-up routine of the Japanese returnees and foreign visitors to Japan and general practitioners' good understanding of the imported tropical diseases are mandatory for the prevention of tropical diseases in the future.

INTRODUCTION

As the number of Japanese travellers abroad increased recently, patients of imported tropical diseases also increased in number year after year. These imported tropical diseases are found among travellers who were affected with the native diseases and felt sick after their return home. Besides the increase of Japanese travellers abroad, gradual increase of foreign visitors to Japan is also found as a new tendency, as cultural exchange with foreign countries are being encouraged. What has to be taken into consideration with this tendency is that there is a big possibility for people from tropical areas of bringing in some tropical diseases.

Actually, according to the questionaire survey of imported malaria by Nakabayashi *et al.* (1975), 14 out of 155 visitors from abroad were reported as affected

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patients. This fact should imply a number of problems of counterplan against imported tropical diseases. The following is a report of our study on foreign visitors in Japan, for the purpose of studying morbidity of imported tropical diseases among alien people.

Subjects and Methods

The subjects of the study were 532 foreign employees of overseas branch offices, factories and sales offices of Matsushita Electric Co. Ltd., who visited Japan from April 1972 to March 1976 from 33 different countries. The classification of regions follows that of the report by Sassa *et al.* (1976) as shown in Figure 1. They are based on the imported tropical disease distribution chart, A; Africa, B; Asia, C; Pacific Islands, D; Middle and South America and E; Caribbean Islands.

In addition, Africa is divided into subareas such as A_1; Middle Western district, A_2; Middle East and neighbouring islands district, A_3; North and Pacific Islands district, A_4; North Eastern district and A_5; Southern district. Asia is also divided into B_1; Eastern Mediteranean Sea and Middle East Asia district, B_2; India and Pakistan district and B_3; South East Asian district.

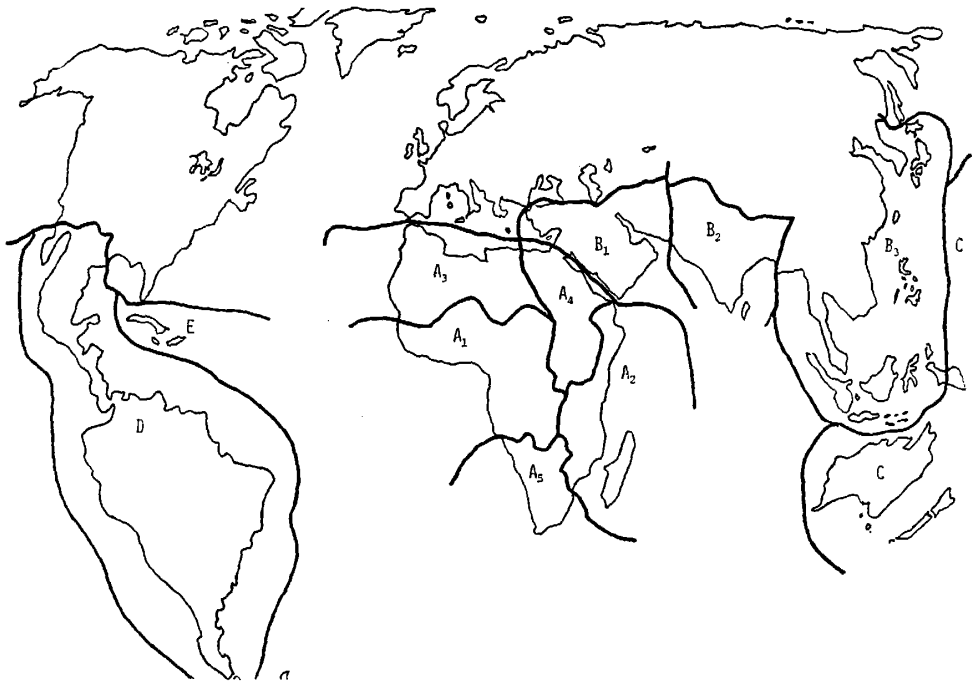

Figure 1 Classification of tropical regions.

The methods of the study were as follows; Examined were people who came for a check-up to the company clinic attached to Matsushita Overseas Training Center, complaining some disorder with in a month after their arrival from the above-mentioned region during the above-mentioned period. All 112 patients were given as much examination as the clinic could offer for the detection of imported tropical

diseases. All 112 patients were examined on their urine. People complaining high fever were given Giemsa staining test of their smear sampling of blood. People complaining diarrhoea were offered microscopic examination for parasites and protozoa and also offered cultural examination for pathogenous microorganisms. Matsushita Overseas Training Center is located in Hirakata City, providing training and housing to foreign trainees in Japan.

Results

1. Regions and countries of foreign visitors and their morbidity rate

All 19 visitors out of A region were from the United Republic of Tanzania from 1972 to 1976. Six patients were found among them and the morbidity rate was 31.6 per cent. Six visitors from C region were all Australians and none was found sick. Of 53 visitors from D region, nine patients were sick and the rate was 17 per cent. The breakdown of D region is as follows; one out of nine visitors from Mexico was sick (11.1%), none of two visitors from El Salvador was sick, two of 10 visitors from Costa Rica were sick (20%), one of two visitors from Panama was sick (50%), one of four visitors from Venezuela was sick (25%), two of 16 visitors from Peru were sick (12.5%), two of 10 visitors from Brazil were sick (20%). Nineteen visitors from E region were only from Puerto Rico and three were found sick (15.8%) (Table 1).

Table 1 Incidence rate in A, C, D and E regions

Group	Country	Year					Total	%	Group-total
		1972	1973	1974	1975	1976			
A_2	United Republic of Tanzania	1/3	1/3	1/4	3/8	0/1	6/19	31.6%	6/19 (31.6%)
C	Australia	0/0	0/2	0/2	0/2	0/0	0/6	0%	0/6 (0%)
D	Mexico	1/3	0/3	0/2	0/0	0/1	1/9	11.1%	9/53 (17.0%)
	El Salvador	0/0	0/0	0/0	0/1	0/1	0/2	0%	
	Costa Rica	0/3	2/6	0/1	0/0	0/0	2/10	20.0%	
	Panama	0/0	0/1	1/1	0/0	0/0	1/2	50.0%	
	Venezuela	0/1	0/1	1/2	0/0	0/0	1/4	25.0%	
	Peru	0/3	0/3	2/7	0/3	0/0	2/16	12.5%	
	Brazil	0/2	0/2	1/5	1/1	0/0	2/10	20.0%	
E	Puerto Rico	3/12	0/6	0/0	0/1	0/0	3/19	15.8%	3/19 (15.8%)

Following are the breakdown of B region. In B_1 district, one of four visitors from Iran (25%) and one visitor from the United Arab Emirates (100%) were found sick. In total, two of five were sick (40%). In B_2 district, three out of nine visitors from India were sick (33.3%). In B_3 district, found sick were nine out of 24 vistiors from the Republic of Korea (37.5%), 15 out of 100 from the Republic of China (15%), six out of 35 from the Philippines (17.1%), five out of 37 from Indonesia (13.5%), two from Hong Kong (100%), one out of nine from Vietnam (11.1%), 13 out of 26 from Thailand (50%), 21 out of 104 from Malaysia (20.2%)

Table 2 Incidence rate in B region

Group	Country	Year					Total	%	Group-total
		1972	1973	1974	1975	1976			
B_1	Iran	0/0	0/2	0/0	1/2	0/0	1/4	25.0%	2/5 (40.0%)
	United Arab Emirates	0/0	0/0	0/0	0/0	1/1	1/1	100%	
B_2	India	0/0	1/1	2/6	0/2	0/0	3/9	33.3%	3/9 (33.3%)
B_3	Republic of Korea	0/0	4/12	1/1	4/11	0/0	9/24	37.5%	75/374 (20.1%)
	Republic of China	2/11	4/23	3/25	2/21	4/20	15/100	15.0%	
	Philippines	1/6	2/10	1/7	2/10	0/2	6/35	17.1%	
	Indonesia	1/3	2/19	1/13	1/3	0/0	5/37	13.5%	
	Hong Kong	0/0	0/0	1/1	1/1	0/0	2/2	100%	
	Vietnam	0/0	0/3	0/5	1/1	0/0	1/9	11.1%	
	Thailand	3/5	0/2	3/6	7/11	0/2	13/26	50.0%	
	Malaysia	5/22	7/32	6/33	2/12	1/5	21/104	20.2%	
	Singapore	2/30	0/2	1/4	0/0	0/1	3/37	8.1%	

and three out of 37 from Singapore (8.1%). Seventy-five of all 374 visitors from B_3 district were found sick and its incidence rate was 20.1 per cent (Table 2).

Forty-seven forigners who came from other regions showed following results. In Europe, one out of three visitors from Spain (33.3%), five out of 12 from West Germany (41.7%), one out of four from Netherland (25%) and one out of three from Denmark (33.3%) were found sick. Three Belgian and three British were healthy in the observation period. Each one who came from Sweden, Switzerland and Poland were sick. In North America, two out of five visitors from the United States of America and one out of 11 from Canada were found sick. Fourteen patients were found out of 47 visitors from other regions (29.8%) (Table 3).

Summerizing the above results, the number of patients out of 485 visitors from 22 countries in the tropical regions of A, B, C, D and E was 98, and its incidence rate was 20.2 per cent, while that of 47 visitors from 11 countries in other region was

Table 3 Incidence rate in other region

Country	Year					Total	%	Group-total
	1972	1973	1974	1975	1976			
Spain	0/0	0/0	0/1	1/2	0/0	1/3	33.3%	14/47 (29.8%)
West Germany	2/3	1/5	1/2	1/2	0/0	5/12	41.7%	
Belgium	0/0	0/0	0/0	0/1	0/2	0/3	0%	
Netherland	0/1	0/1	0/1	1/1	0/0	1/4	25.0%	
United Kingdom	0/0	0/0	0/0	0/1	0/2	0/3	0%	
Denmark	1/2	0/0	0/0	0/1	0/0	1/3	33.3%	
Sweden	0/0	0/0	0/0	0/0	1/1	1/1	100%	
Switzerland	0/0	0/0	1/1	0/0	0/0	1/1	100%	
Poland	1/1	0/0	0/0	0/0	0/0	1/1	100%	
United States of America	0/0	0/3	0/0	2/3	0/2	2/5	40.0%	
Canada	0/1	0/2	1/4	0/2	0/2	1/11	9.1%	

14 and its incidence rate was 29.8 per cent. The total number of patients out of all 532 visitors from 33 countries was 112 and its incidence rate was 21.1 per cent. As seen above, no paticular region, district or country showed specific characteristics in its incidence rate (Table 4).

Table 4 Incidence rate in all regions

Total 33 countries 532 persons 112 patients 112/532 (21.1%)	Tropical area 22 countries 98/485 (20.2%)	A-group 1 country 6/19 (31.6%)	A_2-group 1 country 6/9 (31.6%)
		B-group 12 countries 80/388 (20.6%)	B_1-group 2 countries 2/5 (40.0%)
			B_2-group 1 country 3/9 (33.3%)
			B_3-group 9 countries 75/374 (20.1%)
		C-group 1 country 0/6 (0%)	
		D-group 7 countries 9/53 (17.0%)	
		E-group 1 country 3/19 (15.8%)	
	Other area 11 countries 14/47 (29.8%)	Other country-group 11 countries 14/47 (29.8%)	

2. Classification of diseases

Out of six patients from Tanzania in A_2 district, A region, five were with common cold and one with colitis. In D region, one Mexican was with gastritis, one Costa Rican was with common cold and the other with atopic dermatitis of arms and legs, one Brazilian, one Panaman, one Venezuelan and two Peruvian were with common cold, and one Brazilian with colitis. All three Puerto Rican patients were with common cold (Table 5).

A 31-years-old Iranian from B_1 district, B region, flew to Japan, already suffering from remittant fever since three days before his arrival. On the arrival at Osaka Airport, although he felt fatigue and had fever associated with left parotid swelling, he passed the quarantine inspection, and his fever went down to 37.5 C after a rest on the following day. On the next day (the 3rd day after arrival), the fever went

Table 5 Classification of sickness in each country

A, B, C, D, E-Group

A_2 United Republic of Tanzania (6/19)
Common cold 5 (26.3%)
Colitis 1

B_1 Iran (1/4)
Orchitis 1

United Arab Emirates (1/1)
Common cold 1 (100%)

B_2 India (3/9)
Common cold 1 (11.1%)
Urticaria 1
Angular stomatitis 1

B_3 Republic of Korea (9/24)
Common cold 5 (20.8%)
Motion sickness 1
Menorrhalgia 1
Aphtha 1
Neurosis 1

Republic of China (15/100)
Common cold 12 (12.0%)
Colitis 3

Philippines (6/35)
Common cold 6 (17.1%)

Indonesia (5/37)
Common cold 5 (13.5%)

Hong Kong (2/2)
Common cold 1 (50.0%)
Gastritis 1

Vietnam (1/9)
Asthma 1

Thailand (13/26)
Common cold 8 (30.8%)
Conjunctivitis 1
Colitis 2
Gastritis 1

Malaysia (21/104)
Common cold 19 (18.3%)
Gastritis 1
Tapeworm infestation 1

Singapore (3/37)
Common cold 2 (5.4%)
Urticaria 1

C Australia (0/6)

D Mexico (1/9)
Gastritis 1

El Salvador (0/2)

Costa Rica (2/10)
Common cold 1 (10.0%)
Dermatitis 1

Panama (1/2)
Common cold 1 (50.0%)

Venezuela (1/4)
Common cold 1 (25.0%)

Peru (2/16)
Common cold 2 (12.5%)

Brazil (2/10)
Common cold 1 (10.0%)
Colitis 1

E Puerto Rico (3/13)
Common cold 3 (15.8%)

Other country-Group

Spain (1/3)
Renal calculus 1

West Germany (5/12)
Urethritis 1
Otitis externa 1
Callosity 1
Trauma 2

Belgium (0/3)

Netherland (1/4)
Gastritis 1

United Kingdom (0/3)

Denmark (1/3)
Common cold 1 (33.3%)

Sweden (1/1)
Urticaria 1

Switzerland (1/1)
Panaritium 1

Poland (1/1)
Common cold 1 (100%)

United States of America (2/5)
Colitis 1
Otitis media 1

Canada (1/11)
Common cold 1 (9.1%)

up again to 39 C. An antifebrile and an ice compress of left cheek did not calm it down and on the fourth day after arrival (the 7th sick day), he suffered from orchitis on both sides. Protozoa was not proved by smear sampling of blood. Exanthema and digestive disorder were not found either. Moreover, even after detailed check-up, none of typhoid fever, smallpox, dengue fever, or orchitis usually following malaria was found. Therefore, the case was diagnosed as acute orchitis following acute parotitis, and the patient was hospitalized in Hirakata Municipal Hospital for five days.

One from the United Arab Emirates had common cold. Three Indians from B_2 district had urticalia, angular stomatitis and common cold respectively. Among Korean patients from B_3 district, one was homesick, one with menorrhalgia, one with aphtha, one with neurosis and five others with common cold. Three Taiwanese were with colitis, 12 others were with common cold, one Singaporean was with urticaria and two others had common cold. Six Philippines and five Indonesians were with common cold. One Chinese from Hong Kong was with gastritis and the other was with common cold. One Vietnamese was with atopic asthma and the antigen was suspected as house dust according to anamnesis and intractutaneous test. One Thai was with colitis, one with conjuctivitis, one with gastritis, and eight others were with common cold. One Malaysian was with gastritis, 19 with common cold and with one *Tenia saginata*. This patient caused by *Tenia saginata* was a 27-years-old man and had repeated diarrhoea for one month. He had a habit of eating raw beef. He felt uneasy around the anus 3 days after his arrival to Japan. After the examination, *Tenia saginata* was suspected, he was administered helminthic, 10 grams of *Extractum filicis* and 5 grams of magnesium sulfate in hunger, and 4 meters of helminth was excreted and no diarrhoea was found after that.

In other regions, one Spanish man suffered from right nephrolith and excreted sesame size of calculi after a drug therapy in 18 days of hospitalization. One patient from West Germany was with urethritis, one with right otitis externa, one with callosity and two with trauma. One from Denmark, one from Canada and one from Poland were with common cold, and one Dutch was with gastritis, one Swiss with panaritium, one American with right otitis media and another American with colitis. Eight colitis patients of one Tanzanian, one Brazilian, three Taiwanese, two Thais and one American were consulted with a complaint of one to three times of diarrhoea a day. The examination of parasites of stool and culture of pathogenic microorganisms were all negative (TCBS and SS medium was applied for stool culture) (Table 5).

As mentioned above, no significant relationship was found between the country and patient sample according to these classification of region-countries and patient sample. Among these patients, common cold was found in 77 cases. More people from A, B, C, D and E regions were found with common cold (74 out of 485; 15.3%) than those from North America and Europe (3 out of 47; 6.3%). According to the study of onset of common cold in these patients, most visitors from tropical zone were affected within a week of the arrival and the peak was the third day after their arrival (Fig. 2). Fortunately, every patient recovered by medication, mainly antipyretics and antihistamine, and good rest. Two out of 12 Taiwanese, three out of 19 Malaysians, two out of eight Thais, one out of five Indonesians, one out of

five Tanzanians, one Venezueran who were affected with common cold had fever of at least 37.5 C to 38.5 C. On the patients who were suspected to have had malaria, smear sampling of blood was made, and Giemsa staining test was performed. However, there was found no malaria protozoa shwoing ringstage, segmenter and gametocyte. Therefore, all patients were diagnosed as common cold. Tests for albuminuria and urine urobilinogen did not reveal any significant change.

Table 6 indicates monthly statistics of onset of common cold, in which most of the patients were found in the fall and winter. The upper row indicates the number of patients and the lower the number of foreign visitors in each month. This shows apparently higher morbidity rate of the visitors from tropical zone than those from Europe and North America.

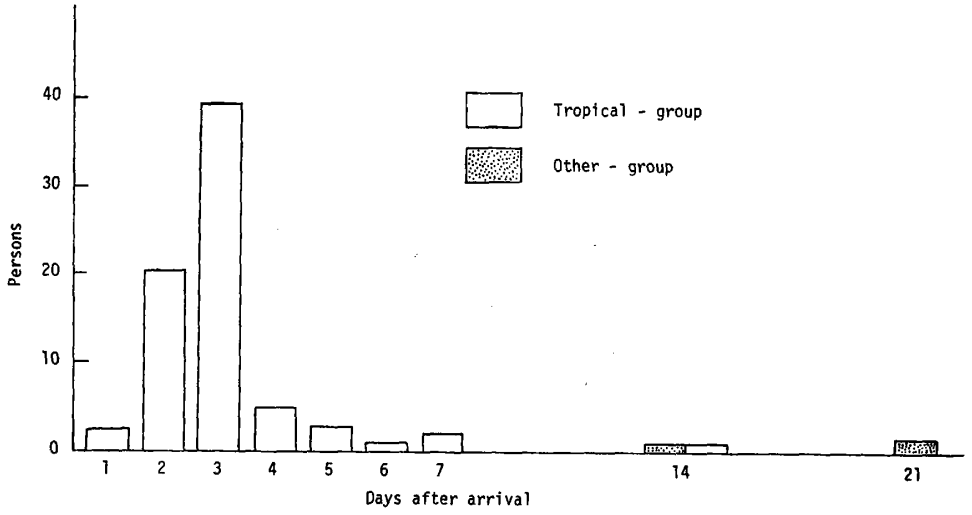

Figure 2 Number of day to onset of common cold after the arrival.

Table 6 Number of total visitors and common cold patients in each month for 5 years

Group	Patients and visitors	Month											
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
A, B, C, D and E group	Common cold patients	6	6	6	4	7	0	0	0	2	18	20	5
	(%)	22.2	24.0	9.4	6.3	11.3	0	0	0	1.2	10.3	13.5	6.3
	Total visitors	27	25	64	64	62	68	75	127	166	174	148	80
Other country group	Common cold patients	0	0	1	0	0	0	0	0	0	1	1	1
	(%)	0	0	10.0	0	0	0	0	0	0	6.3	10.0	20.0
	Total visitors	7	5	10	5	4	2	3	3	8	16	10	5

Discussion

Not only sea men returning from abroad, but also people coming back from abroad and visitors to Japan by air are increasing tremendously in number, owing to increased overseas activities such as sightseeing, business trip, trade, academic

research and study, technical aid and enterprises; more than 10,000 people are coming into Japan everyday through both Tokyo and Osaka airports.

These people usually do not have enough knowledge on prevention and therapy for tropical diseases. Their information is so limited that they get easily affected and some of them died as reported by Ebisawa *et al.* (1973). Imported tropical diseases found after the return are mainly malaria as reported by Ebisawa *et al.* (1969), Tsubura *et al.* (1973), Ebisawa *et al.* (1973), Yamaguchi *et al.* (1973) and Ebisawa *et al.* (1975).

Ebisawa *et al.* (1973) reported a detailed summerization of 73 patients found in 8 years from 1966 to 1973. According to this report, most of the patients had *Pl. vivax*, *Pl. falciparum* and others had with *Pl. ovale* or *Pl. malariae* and were obliged to return home. Diseases other than malaria are also imported. They are nematodiasis, as reported by Kamegai *et al.* (1976), smallpox from Bangladesh, which was fortunately prevented from the 2nd affection in Japan by an appropriate protection at quarantine as reported by Hino *et al.* (1973), and dengue fever reported by Iwamoto (1973). In addition to these continuous reports of imported diseases, recent reports of cholera found in Arita city, Wakayama, and cholera patients found in Kobe who returned from on-board college gives us a warning that we, general practitioners, should be always on the alert of these facts in our day to day check-ups. The report of Kobari *et al.* (1973) on pest in Vietnam implies a possibility of other diseases imported and brings up problems of inspection, early discovery and treatment and prevention of the 2nd affection.

As we had an opportunity of examining 112 foreign patients from 33 countries visiting for job orientation at Matsushita Electric Co. Ltd. for 5 years from 1972 to 1976, we made a survey on medical records.

As shown in Table 4, visitors from A, B, C, D and E tropical regions showed 20.2 per cent of morbidity rate (98 out of 485 visitors), while visitors from the other regions showed 29.8 per cent (14 patients out of 47 visitors). Fortunately we did not find patients with protozoa diseases such as malaria and amebic dysentheria, bacterial diseases such as cholera, typhoid fever, and virus diseases such as dengue fever, smallpox and yellow fever. Unusual cases were patients with *Tenia saginata* and a case with orchitis due to mumps infected before coming to Japan. *Exractum filicis* was prescribed for the *Tenia saginata* patient to excrete helminth, and the orchitis patient was treated with antifebrile and intravenous infusion, antibiotics for protection from of the 2nd affection in the hospital, and both of them were cured.

Another significant finding of our research was the fact that many common cold patients were found among visitors from tropical regions. According to the monthly statistics, this shows different tendency from influenza prevalence in Japan and the visitors get affected in fall, especially in October and November quite different from the peak of influenza in Japan (Table 6). Moreover, most of them became sick after a few days of their arrival. There is an assumption that the reason for this fact is due to the difficulty in changing clothes and living customs, and slow adjustment to the new environment of their sweat gland to control body temperature, and fatigue as well as the fact that they are exposed to invasion of pathogenes into the respiratory tract on the visit to a completely different climate. Therefore, we understand that

early treatment and orientation to Japanese environment, especially to clothes, are of vital importance as the preventive means for them who have had difficulty in using their medical facilities in their home countries. Although import of typical tropical diseases by foreign visitors was not found, there should be a possibility of missing some diseases, and our examinations at Health Consultation Room were not enough because checking points were blood test and stool examination only once.

We are convinced that the above mentioned two examinations are integral parts of prevention of imported tropical diseases when we, general practitioners, examine foreign patients in Japan.

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某社来訪外国人の罹病に関する研究

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国外旅行者の数に比例して熱帯性疾患に罹患するものの増加の傾向が，最近の研究によって指摘さ

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れている。この事実はまた，わが国を訪れ，特に一定期間滞在する外国人が熱帯病病原体を移入し，伝染源となり得る可能性を暗示するものである。我々はこの推定を検討するために1972年より1976年に至る５年間，技術訓練のため熱帯諸国ならびに欧州，北米より松下電器産業株式会社への来訪者について，一定期間の罹病状況を診断または医学記録によって分析し，つぎの結果を得た。

1) 総計532名の来訪者のうち485名が熱帯諸国より，また47名がその他諸国よりの来訪者で，その到着時より１カ月間の罹病率はそれぞれ20.2%，29.8%であった。

2) 今回の調査では幸いにも，マラリア，フィラリア，コレラ等の熱帯病は認められなかった。

3) 母国で感染し，来日に際して発病したマレーシア人の無鉤条虫症，イラン人の続発性睾丸炎各１例が特記されるものであったほかは，感冒，胃腸炎，蕁麻疹等一般的な疾患が多かった。

4) 熱帯圏よりの来訪者は，欧州，北米等よりのそれに比較して，特に秋，冬期に到着後２～３日以内にかなり高率に感冒に罹患する傾向が認められた。

5) 以上得られた成績は，外国人による熱帯病移入の可能性の実態を明らかにするとともに，また，これら来訪者の健康管理上の資料として評価され得る。

6) 帰国邦人および外国人来訪者に対する，理解ある検診ならびに実地医家の，輸入熱帯病についての良き認識は，今後ともこれら疾患の移入を防ぐ対策として不可欠である。

日本熱帯医学会雑誌　第5巻　第3，4号　1977 183-189頁

マラリアのプリマキン療法

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昭和52年11月7日　受付

はじめに

プリマキンは8-アミノキノリン剤に属する抗マラリア剤で，再発型マラリアの赤外型原虫（Coatneyら，1953; Gennisら，1954; WHO, 1967, 1969）と熱帯熱マラリア原虫の生殖母体（WHO, 1967, 1969; Rieckmannら，1968; Bermanら，1971）の駆除剤としてその価値が認められている。

ただし本剤は三日熱マラリア原虫の赤外型原虫駆除には常用量で100％有効でないことも知られている（Ho, 1965; Peters, 1970; Clydeら，1977）。

日本では本剤に関する多数の症例を用いた報告は少ない。われわれは前回（海老沢ら，1977）報告した例にさらに9例を加え，1977年10月までに日本人110人に計132回のプリマキン療法を行ったのでその成績を報告する。

研究方法

1．症　例

1977年10月までに著者らがプリマキン療法を行った日本人症例は110人である。表1に示すように感染原虫の種類は三日熱マラリアが最も多く79人で，熱帯熱マラリア18人，両者の混合感染4人，卵形マラリア8人，四日熱マラリア1人である。

感染地域は東南アジア，インド亜大陸（スリランカを含む）が併せて63人，オセアニア，パプアニューギニア29人，アフリカ17人，南アメリカ1人となっている。

アフリカで感染した三日熱マラリアはエチオピアおよびエジプトそれぞれ1人ずつである。

性別では表2に示すように男103人，女7人で男が圧倒的に多い。

2．薬　剤

主としてWinthrop社製のプリマキンを用いた。1錠中プリマキン塩基として15mgを含む（リン酸プリマキンとして26.5mg含有）。

一部の症例にはCamoprima®（1錠中Amodiaquine 150mgとプリマキン塩基15mg含有）を用いた。いずれも1日1錠ずつ与えた。

14日間連日内服をもって1コースの治療とした。

Table 1　The parasite species and the continent or the area of infection of malaria patients treated with primaquine

Species of malaria parasites*	Southeast Asia and Indian subcontinent	Oceania and Papua-New Guinea	Africa	South America	Total
P.v.	50	26	2#	1	79
P.f.	8	3	7	0	18
P.v.+*P.f.*	4	0	0	0	4
P.o.	0	0	8	0	8
P.m.	1	0	0	0	1
Total	63	29	17	1	110

* *P.f.*=*P. falciparum*, *P.m.*=*P. malariae*, *P.o.*=*P. ovale* and *P.v.*=*P. vivax*.

\# One case each of *P. vivax* infection from Ethiopia and Egypt.

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Table 2 The parasite species and the sex of malaria patients treated with primaquine

Parasite species*	Male	Female	Total
P.v.	74	5	79
P.f.	16	2	18
P.v.+*P.f.*	4	0	4
P.o.	8	0	8
P.m.	1	0	1
Total	103	7	110

* See footnote of Table 1.

表3における短期間療法とは，主として熱帯熱マラリア患者の生殖母体の駆除の際行ったものである。多くの場合1日1錠5日あるいは7日間与えた。1例1日 45mg 頓服で与えた例がある。

三日熱マラリアで短期間（7日）与えた例はプリマキンがなかったためである。その他は前報で説明したとおり熱帯熱と三日熱マラリアの混合感染例で生殖母体駆除のため5日間用いたもの2例と，副作用のため12日で中止した1例である。

3. 副作用

プリマキン内服中の患者は全部無熱期で大部分は外来患者であったため副作用に関する調査は内服終了あるいはその中間の時点で行った。

Table 3 The parasite species and the duration and number of primaquine therapy

Parasite species*	Number of patients	Primaquine therapy		
		Full course#	Short term**	Total
P.v.	79	96	1	97
P.f.	18	8	14	22
P.v.+*P.f.*	4	1	3	4
P.o.	8	8	0	8
P.m.	1	1	0	1
Total	110	114	18	132

* See footnote of Table 1.

\# A full course means 15 mg daily for 14 days, and 30 mg daily for 7 days in 2 cases of vivax malaria.

** A short term treatment means daily 15 mg for 12 days or less. It is usually for 5 days in the case of falciparum malaria.

4. プリマキン内服後の再発の有無

プリマキン内服者はマラリア罹患後国内に半年以上の長期滞在者もあったが2-3カ月後には，マラリア罹患後再び流行地に行く者が多かった。したがって正確な失敗率は求められない。失敗例としてここにあげた例は，プリマキン療法後マラリア流行地に渡航しない中に発病した者，あるいは流行地域に戻ったが都市内にのみ滞在し，帰国後再発した例である。

5. 熱帯熱マラリア原虫生殖母体に対するプリマキンの効果

プリマキンを与える前後において毎日定量的に流血中の原虫数を数えた。求められた μl 当たりの原虫数を対数変換して，経時的原虫数の消長を Ricomac X-844 コンピューターに入れて回帰直線を求めた。

この直線に対してプリマキン内服後の生殖母体数の変動を比較して薬剤の効果判定に用いた。

結　　果

1. プリマキン療法後の三日熱マラリア再発

(i) 1コースの治療後再発

感染地域はパプアニューギニア2人，カリマンタン2人，インド1人の計5人。

この中97日後に再発した女性患者はその後流行地に入って再感染しているので，2回目のプリマキン療法で根治できたか否か不明である。173日目に再発した1人とカリマンタンで感染した2人は，再発時に行った2回目のプリマキン療法後再発していない。インドで感染した1人は2回目のプリマキン療法後6月経ってまだ再発していない。

しかし第1回の再発が232日後に起きているのでなお観察を続ける必要がある。

(ii) 2コースの治療後再発

プリマキン療法を2-3カ月間隔で2回行った後再発した例が3人ある。2例はパプアニューギニアで，1例はフィリピンで感染している。いずれも5カ月以上11カ月半におよぶ長い間隔で再発している。3例とも最終回には1日 30mg，7日間のプリマキンで治療した。パプアニューギニアで感染した2例はすでに1年以上を経過して発病していないので根治したと思われる。フィリピンで感染した例は治療後まだ4カ月しか経っていないので更に観察を続ける必要がある。

(iii) 不完全プリマキン療法

規定の2週間に及ばない内服期間であったので再発したとしてもやむをえない。スラウェシで感染した例は2回目の，14日間の内服でその後再発を起こしていない。インドで感染した例は次回の14日間のプリマキン治療後4カ月を経て再発を起こしていないが，1コースの治療後232日以上経って再発している例があるので注意を要する。

12日間のプリマキン内服後再発した1例は再発の治療後2コースのプリマキン療法を行ってなお再発したことは前述した。最終的には1日 30mg，7日間の治療でその後再発を見ていない。

2. 卵形マラリアのプリマキン療法

プリマキンを与えなかった3例中1例は7カ月の間隔をおいて確実に再発している。

他の2例は1例のみ健在という情報が入っているが両例とも詳細はわからない。

プリマキンを使った8例中1例はオランダに行き，1例は使用後2カ月以内であるので除く。残りの6人はプリマキン療法後1～2年以上経っているが再発を見ていない。

Table 4 Vivax malaria patients who developed relapse following primaquine therapy

Primaquine therapy		Number of patients	Area of infection and number of patients	Interval between the last day of fever and relapse
One course[a]		5	Papua-New Guinea (2) East Kalimantan (2) India (1)	97 and 173 days 141 and 142 days Over 232 days
Two courses[b]		3	Papua-New Guinea (2) The Philippines (1)[c]	172 and 316 days 246 days
Short term	5 days	1	Sulawesi (1)	92 days
	7 days	1	India (1)	34 days[d]
	12 days	1	The Philippines (1)[c]	68 days

a: 15 mg daily for 14 days.
b: Repetition of the above regimen 2 to 3 months apart.
c: The same person. He had to stop primaquine therapy after 12 days because of psychiatric disturbance, relapsed after 68 days, and underwent 2 courses of primaquine unsuccessfully. He was finally cured by 30 mg of primaquine for 7 days.
d: This patient's first illness was treated with clindamycin followed by 7 days of primaquine which was suspended halfway because of shortage of the drug.

3. 副 作 用

(i) 消化器系症状

多少の不快感ないし軟便を訴えるものが2-3人あったが，ほとんどの例はプリマキンを14日間内服し得た。途中で中止した例は1例もなかった。

Camoprima®を内服した例では軟便その他の消化器症状が強く，中止せざるをえない例があった。

(ii) 溶血性貧血

日本人には赤血球の先天性酵素欠損症である G-6-PD 欠亡症は極めて稀である（Miwa *et al.*,

1965）ので各例についてその検査をしなかった。臨床的に溶血性貧血の症状を呈する者はなかった。

(iii) 精神，神経系の異常

次にのべる例は上記所見を呈していたとき入院中で，流血中にマラリアの原虫はいなかったがプリマキン内服開始後7日目より37 C 台の微熱があり，12錠内服した日から異常を呈しはじめた。

症例 F. H. 44歳男，熱帯熱マラリアと三日熱マラリアの混合感染

フィリピンで感染。発病1976年7月20日 入院7月26日。当日の流血中マラリア原虫数は熱帯熱マラリア原虫49,200/μl，三日熱マラリア原虫230/μl。7月29日と30日にMP錠（1錠中スルファモノメトキシン500mg，ピリメサミン 25mg 含有）を2錠と1錠内服した。

8月2日より無熱，8月5日よりプリマキン内服開始。8月11日より微熱。37C台。

精神，神経症状の出現。

8月17日，手のふるえ，異常行動を認められる。

8月18日，時と場所の指南力消失。娘の生年月日失念，問いに対して応答がおそく，しばらく考えてから返事する。

指一指，指一鼻テストはよくできない。左足クローヌス陽性。直進，片足起立不能。

8月19日には左足のクローヌス陰性，精神症状改善す。なお微熱は18日まで続き最高38 C であったがこの間流血中マラリア原虫は陰性であった。溶血性貧血の徴候なし。

本例はその後三日熱マラリア再発のためプリマキン療法をさらに3回繰り返した。すなわち10月25日より14日間，1977年3月中旬より14日間，7月4日より1日 30mg 7日間。とくに10月25日よりの内服は入院中に行ったが精神，神経の異常は認められなかった。

したがって本例における1976年8月17と18日の初回プリマキン療法時に見られた精神，神経症状が何に由来するものかは不明である。

4. プリマキンの熱帯熱マラリア原虫生殖母体に対する効果

(i) 無処置例

発病後早期に発熱発作の治療を行った例では，生殖母体数も少なく，1～2週間で消失した例がある。またかなり多数の幼若生殖母体が出現したにも拘らず，比較的早期に陰性化した例もあった。治療が不完全のため発熱発作の治癒が長びき，成熟した生殖母体が多数出現した例において，連日定性的観察を続けた例では，濃塗標本で陰性化するまで40日以上かかった例がある。

図1では横軸にプリマキン療法開始前後の日数，縦軸に血液1μl中の生殖母体数を対数で示してある。治療開始前に，曲線cの症例では最高25,200/μl，曲線dの症例では最高5,100/μlの値を示した。それぞれ12と10日間プリマキンを与えないで観察を続けた。

コンピューターから得られた回帰直線は

c...$\log y = -0.1366x + 4.3870$ となり

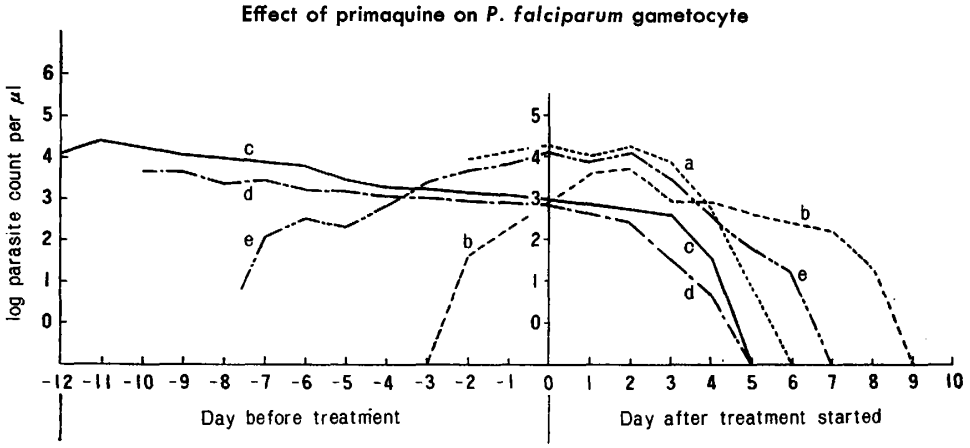

Figure 1 Effect of primaquine on *P. falciparum* gametocyte.

$y=1$ のとき $x=32$ となった。

曲線 d では

d...$\log y=-0.0869x+3.6497$ となり

$y=1$ のとき $x=42$ となった。

すなわち流血中の生殖母体数が0に近づくには，観察初期から数えて1カ月以上かかるということである。$1\mu l$ 中1コ以下とは例えば白血球数が $5{,}000/\mu l$ であるときは，濃塗標本で5,000コの白血球を数えるぐらい視野を移動しても1つも生殖母体が見つからないという数である。

生殖母体が出現しはじめてから次第に増加してゆく例（b, e など）では10^3 あるいは $10^4/\mu l$ 以上に増加してゆくことがわかる。

(ii) プリマキン療法

1日 15mg ずつのプリマキンを与えても治療開始後2-3日間は原虫数にほとんど影響がなく，b例ではむしろ増加してゆく傾向が見える。

しかしながら c, d 例においてはいずれも5日後には陰性となっている。前述の計算では1カ月以上かかることが計算されたので，以上の所見はプリマキンの効力を如実に示すものであろう。

(iii) 無性原虫のクロロキン感受性とクロロキン耐性例におけるプリマキンの反復療法

一般的に言ってプリマキンは，その患者の無性原虫がクロロキン感受性，耐性の如何に拘らず生殖母体に対して効力を示した。プリマキン使用開始後生殖母体消失までの日数は流血中の原虫数の多少にも関係する所見がえられた。

図1において曲線 a, b であらわした例はクロロキン耐性の同一患者の第1回（a）と再燃後の第2回目（b）のプリマキン療法時における生殖母体の消長を示す。第2回目の治療時にはプリマキンに対する反応が遅く，消失までに3日多くかかっている。

以上の所見は同一患者に反復してプリマキンを使用することにより，プリマキン耐性が出現する可能性を示すものか否か，注意を要する所見であろう。

考　　察

1. 再発型マラリアにおけるプリマキン療法の失敗

プリマキン療法を受けた者の中で根治療法が不成功に終った者が何パーセントぐらいあるかは大切な問題であるが，われわれの症例の多くは発熱発作治療後2-3カ月以内にマラリア流行地に行くものがありその答えは得られなかった。

ただし引き続き国内に留っていた例では，再感染のない国内での発病であるから，直ちにこれを再発と見なしうる便利さがある。

パプアニューギニアの三日熱マラリアがプリマキンで根治しにくいことはよく知られている (Peters, 1970)。しかしながらわれわれの症例は1日 30mg 7日間の内服で根治しているので，Chesson 株のように1日 30mg 14日間あるいは1日 60mg 7日間の内服を必要とするほど（Clyde ら，1977）プリマキン低感受性でなかったのは幸である。

プリマキンの1ないし2コースの治療で根治できない三日熱マラリアがさらにフィリピン，カリマンタン，インドなどにもあることは今後これらの地域からの帰国者の治療に際して注目すべきことであろう。1コースのプリマキンで不成功例があることは中国でもすでに報告されている（Ho, 1965)。

2. 副 作 用

プリマキン内服にもとづく溶血性貧血の危険性は常に念頭におくべきであるが，幸にも日本人には G-6-PD 欠亡症が少ないので本剤は安心して使える。

症例 F.H. における精神，神経症状がプリマキン内服によって起こったものか，たまたま同じ頃感染した中枢神経系の感染（日本脳炎あるいは ECHO ウイルス感染症）によるものか不明のままで終わった。後で3回プリマキン療法を行い，3回とも異常を認めなかったので，おそらくはプリマキンによるものではないであろう。

3. 熱帯熱マラリア原虫生殖母体に対する効果

生殖母体が大部分成熟しておりその数値がほぼ一定した，あるいは少しずつ減少しかけて来たとき5-7日間無処置のままで観察すると，対数グラフできれいな回帰直線が求められることがわかった。この式を求めておくと，プリマキンその他の薬剤を用いたとき，その効果判定に役立つであろう。

現在までのところ，無性原虫のクロロキン感受性，耐性の如何に拘らずプリマキンが有性原虫に対して有効であったことは幸である。しかしながら反復使用により感受性低下の傾向も見られるので，再燃を起こさぬよう発熱発作の完全な治療を心がける必要がある。

4．卵形マラリアのプリマキン療法

観察期間が1年以上に及ぶ6人ではすべて再発が起きていないので卵形マラリアに関する限り問題はないであろう。

結　　論

1．プリマキン療法を日本人110人に132回行った。

2．1日 15mg 内服で2週間治療しても大部分の患者は特別な副作用がなく耐えられた。

3．三日熱マラリアではプリマキン1コースないし2コースの治療で根治できない例がパプアニューギニア，カリマンタン，フィリピン，インドから輸入されている。2コースの治療で根治できない例は1日 30mg，7日間内服で根治できた。

4．卵形マラリアは1コースの治療で根治できたと考えられる。

5．熱帯熱マラリア原虫生殖母体はその無性原虫のクロロキン耐性の有無に拘らず，プリマキンで駆除される。反復治療で反応がやや低下した1例があった。

謝　　辞

この研究に用いた Primaquine の一部は石崎達教授（当時国立予防衛生研究所寄生虫部長）の努力により Winthrop 社から供与されたものである。関係者一同に感謝する。

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PRIMAQUINE TREATMENT OF MALARIA

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Received for publication 7 November 1977

Primaquine was given to 110 Japanese malaria patients 132 times for the eradication of *P. falciparum* gametocytes or for the treatment of relapsing malarias.

1) Primaquine was tolerated well by the patients despite minor gastrointestinal discomforts. No hemolytic anemia developed reflecting the extreme rarity of G-6-PD deficiency among the Japanese.

2) The exact failure rate of radical treatment of vivax malaria was not obtained as many patients returned to malaria endemic areas to continue their work. There were two patients each from Papua-New Guinea and Kalimantan and one case from India who relapsed after one course of therapy. Two patients from Kalimantan and one patient from Papua-New Guinea were radically cured by the second course of primaquine treatment. Another case from Papua-New Guinea was reinfected in the same area and another case from India is free from malaria for 6 months after the second primaquine treatment.

Three patients relapsed after two successive courses of primaquine treatment at an interval of two to three months and were radically cured by 30 mg of primaquine given over seven days.

3) All the six ovale malaria patients were radically cured by one course of primaquine treatment.

4) *P. falciparum* gametocytes were sensitive to primaquine irrespective of the chloroquine-resistance of the asexual form of the parasites. There was indication that the response to primaquine in the second treatment was a little slower than in the first treatment.

The gametocytes in the untreated with primaquine patients, after they reached a peak level, decreased logarithmically and their trend was expressed as $\log y = -ax + b$, where x is the day after treatment with schizontocidal drug, y the parasite count per μl of blood, and a and b constants. The equations in two patients were as follows. $\log y = -0.1366x + 4.3870$ and $\log y = -0.0869x + 3.6497$.

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Japan. J. Trop. Med. Hyg., Vol. 5, No. 3, 4, 1977, pp. 191-246

PATHOLOGICAL STUDY ON EARLY CHANGES OF CHOLERA PATIENTS

—Based on 21 Autopsied Materials—

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Received for publication 10 November 1977

Abstract: 1) Morphological observations were made on 21 cholera patients who were autopsied soon after death. Since 19 of the cases were those dying within 41 hours after the onset, the changes with time in the early stage of cholera were studied.

2) Small intestinal mucosal abnormalities are represented by acute inflammatory reactions and subsequent atrophic alteration of villous architecture. In these changes, the increased reproduction of epithelial cells in crypts by inflammatory stimulus seems to play an important role.

3) Increased secretion of excretory glands was seen in goblet cells of the small and large intestines, in Paneth's cells of the intestine, and in goblet cells of the bronchi in cases of aspiration of gastric content. Increased secretion of pancreatic juice and bile was also observed.

4) It is speculated that cholera toxin is absorbed by the intestine or some stimulating substance is produced in the intestine. This is suggested by a) reactions of the mesenteric lymphnodes and spleen, b) fatty changes of the liver and kidney, and c) increased secretion of pancreatic exocrine system.

5) The death may be attributed to cardiac failure and swelling of the brain resulting from dehydration. Respiratory disturbance due to aspiration of gastric content should also be considered. Renal changes in the early stage of the disease are mild and consequent renal failure could have been prevented by proper transfusion.

INTRODUCTION

There have been few reports of pathological studies on systemic organs of autopsied cholera patients except for the recent reports by Sun *et al.* (1971) and Norris (1973). Studies on pathological changes in a specific organ of cholera patients such as the intestine, kidney or adrenal are also scarce.

The present paper is to describe in detail the morphological findings of systemic organs on the basis of autopsy materials, as well as to discuss the pathogenesis and pathophysiology of the changes in cholera patients.

An attempt was made to find the characteristic changes of organs resulting from cholera infection and development of the disease by observing morphological changes of organs with the time after the onset. It was also attempted to study whether or not the changes in systemic organs other than the gastro-intestinal tract which is

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the site of infection can be explained only by dehydration, as well as to verify whether or not cholera toxin or some unknown factor is taken into the body.

The 21 autopsied cholera cases used as materials of this study were characterized by the following.

1) The interval between onset and death was within 41 hours except in two cases with the intervals of 7 days and 3 weeks.

2) Most of the patients were infants and children. Of the 21 cases, 16 cases were at the age of 10 or younger.

3) These cases were autopsied shortly after death (within 2 hours in 20 cases), and consequently the intestine and other organs could provide informations as well as biopsy specimens.

Materials and Methods

Among the patients who died in the cholera ward of the San Lazaro Hospital in Manila between 1968 and 1970, 21 cases autopsied shortly after death were used for this study (Table 1).

The occurrence of diarrhea was regarded as the onset of the disease. The 21 cases consisted of 11 cases of death within 14 hours after the onset, eight cases

Table 1 Autopsy cases of cholera in 1968–1970 in Manila

Autopsy No.	Age	Time after onset	Admission time	Postmortem time	*Vibrio*	Diarrhea	Transfusion	Dehydration
1	45	a few hs.	0: 05	1: 10	+	++	—	+++
2	6	a few hs.	1: 10	4: 00	+	+	2 *l*	+++
3	3	7 hs.	0: 25	0: 45	+	+	—	+++
4	63	8	0: 05	0: 40	+	+++	—	+++
5	6	10	2: 10	1: 25	+	?	1 *l*	+++
6	20	10	3: 20	1: 50	?	++	1 *l*	+++
7	8	10	8: 15	1: 00	+	+++	3.5 *l*	+++
8	2	11	2: 10	0: 50	+	+++	2 *l*	++
9	4	12	2: 10	0: 40	+	+++	1 *l*	+++
10	3	13	0: 30	1: 30	+	++	1/4 *l*	++
11	6 mons	14	11: 00	0: 55	+	+++	1/4 *l*	+
12	65	24	0: 10	1: 10	+	+++	—	+++
13	2	24	18: 00	0: 50	−	?	?	+++
14	5	30	24: 00	1: 05	−	+++	1.6 *l*	±
15	3	32	29: 00	2: 40	+	+++	?	±
16	4	33	28: 00	1: 20	+	+	2 *l*	+++
17	8	40	35: 30	1: 00	−	+++	2 *l*	+++
18	14	40	38: 00	2: 05	+	+	3 *l*	+++
19	1	41	32: 00	1: 00	+	+	1.5 *l*	±
20	2	7 ds.	0: 00	1: 45	+	+	—	+++
21	6 mons	3 ws.	3 ws.	0: 50	?	+	?	+++

during 24–41 hours and one case each of 7 days and 3 weeks. As classified by age, 16 cases were infants and children at age 10 or younger, two cases were at age 60 or older, and three cases were between age 10 and 60. Frequent and voluminous diarrhea and vomiting were observed in all the cases. At the time of death, 17 cases showed severe dehydration.

Fever of 39–40 C was noted in seven infants and children. Transfusion of lactated Ringer's solution and administration of antibiotics were provided according to the degree of dehydration after the admission. However, therapy was not provided for the cases of death soon after the admission.

Bacteriological examination using rectal swab or intestinal content obtained at autopsy was positive in 16 cases, negative in three cases and was not performed in two other cases, in which, however, typical symptoms of cholera were manifest.

Efforts were made to perform autopsy as soon as possible after death and to open the intestine for prompt fixation in order to prevent post-mortem changes, particularly autolysis of the intestinal mucosa. In fact, all the cases in this study were autopsied within 4 hours after death. The blocks of tissues resected at autopsy were fixed in Zenker-formalin solution and 10 per cent formalin solution, and tissue sections were stained by ordinary stains and special staining methods for specific organs and specific purposes.

Results and Comments

SMALL INTESTINE

Macroscopic findings:

Macroscopic findings described in autopsy protocols were available for 18 cases (Table 2). Distension was noted in the cases of death over 24 hours after the onset. In typical cases, a large amount of watery content, moderate edema of the intestinal wall and advanced anemia of the mucosal surface were observed. The mucosal surface was thickly covered with dense mucus in most cases. The intestinal content was, at the early stage after the onset, turbid in whitish color mixed with mucus appearing as so-called rice-watery stool, but with the lapse of time, it became thick and the color turned to bile-stained green and yellow. Blood components were contained in one case (No. 12). Ascaris and small roundworms were observed in many cases. Ascaris occasionally formed a mass and the adjacent mucous membrane was hyperemic.

Dissecting microscopic findings:

The dissecting microscopic pictures of the mucous membrane resected from the upper and lower parts of the jejunum and the lower part of the ileum of 18 cases were examined and photographed, and consequently the appearance of villi was classified into five types (Table 2).

I: finger-like II: tongue-shaped III: leaf-like

IV: ridge-formation (Photo. 1) V: convolution (Photo. 2)

There was no case of completely normal appearance. Even in type I, the villi

were swollen showing cobblestone-like appearance (Photo. 3). The appearance of villi in cholera is considered to represent atrophy-like changes being essentially the same as the changes of coeliac disease and non-tropical sprue. Deformity of villi was more frequent in the jejunum than in the ileum and more remarkable in the ridge of circular folds than in the vallecula. Between the cases dying within 14 hours and the cases dying after 24 hours atrophic changes were more remarkable in the latter cases, suggesting that cholera is a progressive disease.

Table 2 Macroscopic and dissecting microscopic findings of small intestine

No.	Age	Time after onset	Distension of intestine	Anemia of mucosa	Color of content	Ascaris	Jejunum	Lower jejunum	Ileum
2	6	a few hs.	−	++	white	many	IV		Ic
3	3	7	++	++	yellow	9	IV	IV	III
4	63	8	−	−	white	6	IVc	Ic	Ic
6	20	10	+	++	white	15	IIc	IIc	Ic
7	8	10	−	++	white	58	Vc	Vc	IIIc
9	4	12	−	−	white	9	V	V	IV
10	3	13	−	−	white	15	IIIc	IV	IV
11	6 mons	14	++	++	yellow	−	V	IIIc	III
12	65	24	−	−	reddish	1	IV	IIIc	IIIc
13	2	24	++	+	green	5	V	V	IIIc
14	5	30	++	−	green	30	IV	Ic	Ic
15	3	32	−	+	yellow	90	V	IV	IIIc
16	4	33	+	+	green	25	IV	III	III
17	8	40	−	+	yellow	many	V	IIc	Ic
18	14	40	+	±	green	20	IV	IIIc	IIIc
19	1	41	++	+	green	−	IV	V	IVc
20	2	7 ds.	−	++	white	−	V	V	IIIc
21	6 mons	3 ws.	++	+	green	−	V	V	V

I: finger-like, II: tongue-shaped, III: leaf-like, IV: ridge-formation, V: convolution, c: cobblestone-like

Histological changes:

The histological changes of the small intestine were noted mostly in the mucosa and were classified into two major types. In the first type, the basic architecture of the mucosa was maintained but severe inflammatory cell infiltration of the lamina propria and advanced desquamation and sloughing of the epithelial cells of villi were noted (Photos. 4, 22). The second type involved architectural alteration of villi together with blunting and fusing, resulting in the appearance of atrophy (Photos. 5, 8, 14). The former was common in cases within 14 hours and the latter in cases over 24 hours.

i) Changes of intestinal epithelial layer

No marked degeneration and necrosis were noted in the epithelial cells, but cytoplasmic vacuolization of the upper half of villi was noted in one case (No. 11) (Photos. 6, 7). Edematous slit adjacent to the basement membrane was seen occasionally (Photo. 8). Although some reports imposed a significance to this change,

it seemed to be a postmortem change or an artifact change during the preparation (fixation) of specimens, since this change is seen also in the intestine of usual autopsy other than cholera. As a postmortem change, desquamation and sloughing of the epithelium are also noted in usual autopsy materials. In the present study, this change was advanced (Photo. 22) in cases within 14 hours and slight in cases over 24 hours (Table 3). In consideration of the fact that the subjects of this study were autopsied within a short time after death, the epithelium must have been in the state to readily slough in the cases within 14 hours. Normal villous epithelium being 20–30 μ in height is taller than the cryptal epithelium but the height in cholera cases was reduced to 16.8 μ in mean being shorter than the cryptal epithelium. The epithelium in severely atrophic villi was cuboidal or flat (Photo. 9). The brush border of the epithelium was preserved by PAS stains (Photo. 10). In the cases with marked inflammatory cell infiltration of the lamina propria, the epithelium was invaded by lymphocytes (Photos. 7, 9).

ii) Changes in lamina propria

The lamina propria was swollen by inflammatory cell infiltration and the villi as a whole became blunting. Hyperemia was not noted. Only No. 12 showed

Table 3 Epithelial changes and villous fusion of small intestine

No.	Age	Time after onset	Desquamation of epithelium	Degeneration of epithelium	Height of epithelium Villus (μ)	Crypt (μ)	Villuos fusion Epithelial	Proprial
1	45	a few hs.	−	−	19	21	−	+
2	6	a few hs.	++	+	14	22	−	−
3	3	7	++	−	19	22	−	−
4	63	8	++	−	14	18	−	−
5	6	10	++	−	19	21	−	−
6	20	10	+	−	10	11	+	+
7	8	10	++	−	19	23	+	+
8	2	11	−	−	18	19	−	−
9	4	12	++	−	15	23	−	−
10	3	13	++	−	17	21	−	+
11	6 mons	14	−	−	15	20	−	+
12	65	24	?	?	17	19	?	?
13	2	24	−	−	13	20	+	+++
14	5	30	±	++	14	20	+	++
15	3	32	+	−	19	22	+	−
16	4	33	+	−	18	23	−	−
17	8	40	±	+	18	22	+	++
18	14	40	+	−	20	23	+	−
19	1	41	−	−	18	20	+	++
20	2	7 ds.	−	−	21	22	+	+++
21	6 mons	3 ws.	−	−	15	19	+	+

hemorrhagic enteritis. The central lacteal showed dilatation in part (Photo. 10) but this was not characteristic. Inflammatory cell reaction was strong in the cases within 14 hours (Table 4). Plasma cells were dominant (Photo. 11) with minimal neutrophils in most cases but lymphocytes were dominant in some cases. Histiocytes were increased in the cases over 24 hours (Photo. 12). In some of the cases within 14 hours, focal invasions of eosinophils were noted but there was no relation to the parasitism of ascarides.

iii) Atrophy of villi

Three sections each of the jejunum and ileum were obtained from the cases for which the entire intestine was preserved upon formalin fixation, and histological measurements were performed (Table 5).

The average mucosal height was 510 μ in cases within 14 hours and 442 μ in cases over 24 hours, the latter being reduced by 13 per cent. The height of the villus was 294 μ in cases within 14 hours and 206 μ in cases over 24 hours showing a reduction by 30 per cent, and the depth of the crypt was 182 μ in the former group and 212 μ in the latter group with an increase by 16 per cent. The mean value of ratio of villous height and cryptal depth was 1.6 in cases within 14 hours and 1.0 in cases over 24 hours. In comparison with the cases within 14 hours, the cases over

Table 4 Infiltration of inflammatory cells to lamina propria

No.	Age	Time after onset	Severity of infiltration	Type of cell			
				Lympho-cyte	Plasma cell	Histio-cyte	Eosinophil (focal)
1	45	a few hs.	++	+	+		+
2	6	a few hs.	+++		+++	++	+
3	3	7	+	+	++	+	+
4	63	8	+	++	+	+	+
5	6	10	++	++	++		
6	20	10	++	+	++		++
7	8	10	+++		++		
8	2	11	+++	+	++		+
9	4	12	+++	++	+++		++
10	3	13	++		+++	+	
11	6 mons	14	++		+++	+	+
12	65	24	++	+	++		
13	2	24	+	++	+	+	+
14	5	30	++		+	++	
15	3	32	+		++	++	
16	4	33	+		++	+	
17	8	40	+	++	+		+
18	14	40	+	+	+	+	+
19	1	41	+		++	++	
20	2	7 ds.	+		+	+++	
21	6 mons	3 ws.	+	+	+	++	

Table 5 Measurements of villus and crypt

No.	Age	Time after onset	Height of mucosa	Height of villus	Depth of crypt	Villus/Crypt	Number of mitosis in 10 crypt
1	45	a few hs.	510	294	182	1.6	10.5
2	6	a few hs.	486	254	180	1.4	20.6
3	3	7	710	464	226	2.1	7.7
4	63	8	456	296	164	1.8	4.7
5	6	10	510	294	182	1.6	7.3
6	20	10	400	140	240	0.6	3.2
7	8	10	440	248	172	1.4	8.9
8	2	11	510	350	140	2.5	7.8
9	4	12	516	320	176	1.8	8.1
10	3	13	520	346	156	2.2	5.3
11	6 mons	14	560	236	192	1.2	7.1
	Average		510	294	182	1.6	
12	65	24	476	296	160	1.9	3.3
13	2	24	440	190	230	0.8	12.0
14	5	30	416	96	300	0.3	12.3
15	3	32	496	240	236	1.0	8.1
16	4	33	484	344	120	2.9	4.7
17	8	40	456	236	200	1.2	6.0
18	14	40	626	316	290	1.1	12.0
19	1	41	424	188	216	0.8	7.0
20	2	7 ds.	364	96	220	0.4	5.0
21	6 mons	3 ws.	236	64	152	0.4	3.4
	Average		442	206	212	1.0	

24 hours show the following characteristics; (1) the mucosal height is decreased, (2) the height of the villus is decreased, and (3) the crypt is elongated.

iv) Fusion of villi

Fusion of villi which is usually considered characteristic to chronic enteritis was observed in the subjects of this study. Fusion was seen only between the epithelial layers of villi in mild cases (Photo. 13) but it involved the lamina propria in severe cases (Photos. 5, 14). Thus the degree of fusion increased with time as shown in Table 3. The architecture of reticulum fibers was observed by silver impregnation for reticulum. This ranged from essentially normal architecture with broadening of reticulum fibers due to the infiltration of inflammatory cells (Photo. 15) to what is called colonization where reticulum fibers were already increased and communicated with the lamina propria of the adjacent villi (Photo. 16). The villous fusions observed in this study were not all the same. It was understood that some of the fusions had been present prior to the onset and some others appeared after the onset.

v) Number of mitoses in the crypts

The crypts are the sites of mitoses of epithelial cells and elongation of crypts was observed in the subjects of this study (Photos. 4, 5, 14). In view of this, the number of mitoses per crypt was measured instead of mitotic index, using 10 crypts sectioned longitudinally exactly at the middle of cryptal lumens from among the six intestinal sections per case (Table 5). The value in the subjects of this study was 0.78 per crypt against 0.5 in normal Japanese, indicating increase of cell division of the epithelial cells.

vi) Goblet cells

Goblet cells as a whole showed increased secretion of mucus and were classified into the following four phases: (1) Enlarged and filled with secretory granules (Photo. 17), (2) secreting, (3) inside becomes reticular with the progress of excretion (Photo. 18), (4) reduced in size upon completion of excretion (Photo. 10). In each villus, the change from (1) to (4) was observed in order from the base to the tip.

The phases (1) and (2) were common in the cases within 14 hours and the phases (3) and (4) in the cases over 24 hours. Most goblet cells of the crypt were in phases (1) and (2), indicating slower reaction (Photo. 19).

vii) Paneth's cells

The secretory granules of Paneth's cells were eosinophilic in Hematoxyline-Eosin stain, and were stained red with azocarmine in Heidenhain's stain and with Ponceau-Fuchsin and Orange G in Masson's trichrome stain.

Excretion of granules were enhanced in most of the cases. Granules were being excreted into the cryptal lumen in some cells and excretion was completed in some other cells becoming empty (Photo. 20). Granules were hardly recognized in the cells of advanced change. Some granules were irregular in size and some others were conglomerate to form coarse granules (Photo. 19). Some cells contained only immature granules that were stained pale blue with Heidenhain's azocarmine stain and light green with Masson's trichrome stain (Photo. 21). These findings were seemed to be the result of enhanced secretion. There was no difference by the course of time. In comparison between regions, degranulation was somewhat advanced in the jejunum than in the ileum.

viii) Lymphoid tissues in intestine

Hyperplasia of solitary lumph follicles and Peyer's patches were seen in most of the infants and chidren (Photo. 22). Reaction was strong in the ileum but hyperplasia of lymph follicles was also remarkable in the duodenum and the jejunum. Follicles were swollen and the margin became indistinct. Nuclear debris in the hyperplastic germinal center was also noted (Photo. 23). The changes in adult cases and infant cases of 7 days and 3 weeks were minimal.

Comment on small intestine:

Morphological studies on the intestinal mucosa of cholera patients were originated by an autopsy report by Virchow (1879). He considered that the epithelium of intestinal villi was denudated and the serum leaked from the lamina propria of the mucosa, resulting in voluminous diarrhea. This concept was supported by Koch (1892) and Deycke (1892) and had been believed to be correct for about 80 years. However, Cohnheim (1889) and Goodpasture (1923) thought that denudation of the

epithelium was rather a postmortem change.

Gangarosa *et al.* (1960) performed intestinal biopsy in cholera patients using an apparatus devised by Crosby (Shiner, 1957) and demonstrated that the intestinal epithelium of cholera patients was not denudated. He further described that the histopathological abnormality of the small intestine in cholera patients was acute enteritis manifested by mononuclear cell infiltrantion, vascular congestion, marked hyperplasia of goblet cells with eventual exhaustion atrophy and increased turn-over of epithelial cells. He attributed atrophic alteration of villi in cholera to the superimposition of acute inflammation due to cholera on chronic atrophic enteritis due to predisposing dietary and nutritional factors in individuals in epidemics. This concept that acute inflammation due to cholera is superimposed on chronic enteritis is generally supported in Western countries (Fresh *et al.*, 1964). Recently, Pastore *et al.* (1976) reported that his histological biopsy study of cholera patients during an epidemic in Southern Italy revealed no chronic sprue-like enteropathy in Western cholera patients.

Certainly many investigators have reported that atrophy of villi is observed even in individuals without diarrhea in tropical regions where cholera may be prevalent (Russell *et al.*, 1966; Chacko *et al.*, 1969). Stransky and Dizon-Santos-Ocampo (1957) found severe atrophy of the intestinal mucosa at autopsy of Philippinos with malnutrition. Sprinz *et al.* (1962) examined intestinal biopsy specimens of Thailanders and reported that the intestinal mucosa of Thailanders of the lower socioeconomic stratum showed changed which were essentially the same as the changes in patients of non-tropical sprue and idiopathic steatorrhea in Western countries, and that the morphologic findings of the intestine in patients who had had the disease one year previously and recovered from the disease were similar to the findings of other Thai people who had never had cholera or any known gastrointestinal disease.

Watanabe *et al.* (1970) who reported previouly the morphological abnormalities of the intestinal mucosa in 12 patients included in this study, first classified the alteration of villi into the following three stages.

First stage: stage of inflammation — Inflammatory cell infiltration is marked in the small intestinal mucosa.

Second stage: Stage of villous fusion — Blunting and fusing of villi dominate the histological scene resulting in a tremendous decrease of surface area, while inflammation in the lamina propria is subsiding.

Third stage: villous resolution — Fused villi are again spearated.

According to the relation between the time course of the disease and the features of villous alterations, he paid attention to epithelial fusion observed in the second stage and postulated that the fusion was an acute change. As the materials consisting with the third stage were not obtained, this stage is still a theoretical one.

In the present study with nine additional cases those in Watanabe's report, alterations were classified into the first stage and the second stage. In comparison of the cases within 14 hours and over 24 hours, reduced inflammation, flattened epithelium, atrophy of villi and elongation of crypts were observed in the latter group, and these alterations were considered to have resulted from cholera.

Sheehy *et al.* (1964) reported that partial villous atrophy was seen in infectious

hepatitis. Collins (1965) and Collins and Isselbacher (1965) demostrated that severe villous atrophy was observed in cases other than celiac disease and tropical sprue, and reported that villous atrophy could be caused by numerous factors. This indicated that, when a stimulus of any cause is applied to the small intestinal mucosa, the intestinal villi may possibly result in atrophy (Gottlieb and Brandborg, 1966; Schenk *et al.*, 1967; Klipstein and Schenk, 1975).

The mitosis of epithelial cells in the intestinal mucosa occurs in the crypt and the regenerated epithelium rises like an escarater on the surface of villus and is ejected from the edge of the villus (Leblond and Walker, 1956; Kaku *et al.*, 1962). The life span of the epithelium of the small intestinal mucosa is very short being 27 to 30 hours in mouse, 48 hours in rat (Altmann and Enesco, 1967) and 2 to 3 days in man. This suggests that advanced alteration of architecture of the small intestinal mucosa may possibly take place during a short period of 2 to 3 days in acute enteritis.

Based on the experimental study by administration of *Salmonella typhimurium* into the intestine of the guinea pig, Kunita (1972) reported that villous shortening, villous adhesion, decrease of the villus-crypt ratio, and atrophic change noted in sprue may possibly occur within a day in acute enteritis.

Abram *et al.* (1963) observed atrophy of villi and elongation of crypts in experimental *Salmonella typhimurium* enteritis, and also confirmed acceleration of epithelial regeneration on autoradiography and concluded that this played an important role in alteration of architecture of the mucosa.

Fujita (1971) has described from the aspect of cell dynamics that, when a certain offensive factor is applied to the villus, extrusion of epithelial cells is accelerated and proliferative reaction of the cells is enhanced; and consequently the crypt becomes hyperplastic and the villus atrophic, and the small intestinal mucosa is covered with immature villous cells and its absorptive function decreases. This theory is useful to explain architectural alteration of the small intestinal mucosa caused by various factors.

Vibrio cholerae does not directly invade the epithelium of villi and consequently the damage to epithelial cells is minimal. However, the enhanced desquamation as observed in the cases within 14 hours leads us to estimate the degeneration in consideration of postmortem changes. Moreover, the advanced inflammatory cell infiltration of the lamina propria suggests a great influence of offensive factors on the intestinal mucosa. The number of mitoses of epithelial cells in the crypt was increased in the subjects of this study.

Kent and Lindenbaum (1967) observed dense infiltration of inflammatory cells in the lamina propria, decreased villus to crypt ratio and cuboidal change in the epithelial cell in cholera patients, which were essentially the same as those of other acute diarrheal disease found in the biopsy specimens in East Pakistan.

Asakura *et al.* (1973) conducted a biopsy study of cholera patients and control subjects at the San Lazalo Hospital in Manila and observed atrophy of villi and elongation of crypts in cholera patients, endorsing the results of examination in the subjects of this study.

Inflammatory cell infiltration of the lamina propria is a finding which is unfailingly described in all reports of autopsy and biopsy materials of the disease (Fresh

et al., 1964). The same finding has been observed also in experimental cholera with administration of *Vibrio cholerae* (Goldstein *et al.*, 1966). There has been a report that inflammation was not caused by the administration of cholera toxin (Steinberg *et al.*, 1975). This indicates the possibility that intestinal changes in cholera cannot be explained only by cholera toxin.

There have been many reports that indicate congestion and hyperemia in the lamina propria and submucosa (Fresh *et al.*, 1964; Norris *et al.*, 1965; Norris and Majno, 1968; Sun *et al.*, 1971). Morishita (1977) observed redness and hyperemia in his endoscopic studies. The subjects in the present study were anemic except for one case (No. 12) with bleeding of the mucosa.

Increment of permeability of blood vessels has been known as another important biological activity of cholera toxin (Ghosh and Mukerjee, 1959; Craig, 1970; Kamisaka, 1972). This activity has been observed mostly in the skin (Finkelstein *et al.*, 1966; Richardson, 1969; Hashimoto *et al.*, 1974) but the relation with diarrhogenic activity in the intestine is being discussed (Sprinz, 1962). Increased permeability is not participated by dilatation of vessels but is described as a cytotoxic change of the endothelium (Dalldorf *et al.*, 1969). There has been a report of experimental study that accumulation of fluid in dogs with Thiry-Vella loops was not reduced despite the decrease of blood pressure of the mesenteric artery (Carpenter *et al.*, 1969).

These results indicate that hyperemia or congestion is not required for excretion of water and that anemic intestinal mucosa in some cases of this study is not unreasonable.

Enhanced excretion of goblet cells have been reported on many human and experimental cases (Norris and Majno, 1968; Elliott *et al.*, 1970; Blackman *et al.*, 1974). Since the decrease of goblet cell mucus was seen only in cholera toxin in comparison between shigella toxin and cholera toxin (Steinberg *et al.*, 1975), enhanced excretion of goblet cells may be the effect of cholera toxin. In this study also, enhanced excretion was remarkable and the surface of the mucosa was covered with a thick mucous coat. Increased production and excretion of mucus were observed also in goblet cells of the colonic mucosa and the bronchial mucosa, and these changes were considered to have the same mechanism as in the small intestine, namely cholera toxin.

Paneth's cells were present at the base of crypts and contained in the supranuclear region granules of equal size measuring approximately 1 μ and being eosinophilic in staining. The secretory granules are known to be digestive enzymes but their quality and physiological activity have not been thoroughly elucidated (Hally, 1958). Thurbeck *et al.* (1960) repoted that the number of Paneth's cells increased in tropical sprue, whereas Shiner and Doniach (1960) have found no remarkable change. There have been no report on the number of Paneth's cells and their excretion in cholera, but considerably enhanced excretion was noted in this study.

The intestinal changes in the subjects of this study may be summarized as follows.

1. The villous architecture of the small intestine showed sprue-like changes in the course of time such as shortness of villi, villous fusion and elongation of crypts. In some cases of this study, the sprue-like changes seemed to have been present prior

to the onset of cholera but it was suggested that the changes were intensified during the course of cholera. Cholera is an acute intestinal infectous disease with quite a short course and little epithelial injuries, but the changes resulting therefrom are sprue-like changes.

2. The intestinal epithelium seemed to be degenerated slightly. This was suggested by the intensified epithelial desquamation as the change in agonal and postmortem states. This desquamative change was remarkable in the cases within 14 hours.

3. The inflammatory cell infiltration in the lamina propria involving mostly plasma cells and lymphocytes was remarkable in the cases within 14 hours. The infiltrative cells were then replaced by histiocytes. The small intestine was anemic without congestion and dilatation of blood vessels. Hyperplasia of lymphoid tissue and reaction of germinal center were remarkable.

4. The number of mitoses increased in the crypt. This was considered due to inflammatory stimulation. This resulted in expansion of the proliferative zone, i.e., elongation of the crypt.

5. The exocrine cells such as goblet cells and Paneth's cells showed enhanced secretion.

STOMACH

Macroscopic findings:

Dilatation was noted in half of the cases and the internal cavity was filled with gas, watery content and sometimes coffee ground-like material. There was no erosion or ulcer and the mucosa was pale.

Histological findings:

Histological examination of the stomach was performed on 17 cases. Edema of the lamina propria and submucosa, and inflammatory cell infiltration were not observed (Photos. 24, 25). Slight desquamation of the mucous epithelium was seen in half of the cases. Mucous cells of the epithelium showed enhanced excretion (Photo. 24). Probably because of the enhanced excretion of zymogenic granules in the chief cells, coarse density of intraplasmic zymogenic granules was noted in 60 per cent of the subjects, and such granules almost disappeared in four severe cases. Parietal cells showed large vacuoles in the cytoplasm in six cases. Particularly in severe cases, the cytoplasm was almost vacuolated (Photo. 26). Changes were intensive in the neck portion of the gastric gland. Atrophic change of the mucosa was noted in one (No. 4, age 63) of the two senile cases but not in the other case (No. 12, age 65) with hemorrhagic gastritis.

LARGE INTESTINE

Macroscopic findings:

Trichuris was seen in 10 cases. Meteorism (gas containing) was noted in four cases and marked dilatation or relaxation of the colonic wall in five cases. The

content ranged from rice-water like fluid to that mixed with bile and occasionally with minimal blood coagula. The mucosa was covered with white mucus, and in some cases, the content was scarce. The mucosa was pale except in four cases with hyperemia.

Histological findings:

The main changes were increased secretion of goblet cells with slightly increased desquamation of the epithelium of the free surface of the mucosa. The epithelial desquamation was more intensive in the cases within 14 hours. Goblet cells were either filled with mucus (Photo. 27), marked with secretion together with dilatation of the gland (Photo. 29), or reduced in size upon completion of secretion (Photo. 29). Round cell infiltration of the lamina propria was more intensive (Photo. 29) in the cases within 14 hours. There was no infiltration of neutrophils. In case No. 20 (7 days), acute colitis with features such as dilatation of glands, formation of cryptal abscess and severe inflammatory reaction of the lamina propria was noted.

Comment on stomach and large intestine:

Concerning findings of the stomach in cholera, no change is reported in some reports and mild inflammatory reaction in some others (Gangarosa *et al.*, 1960; Pastore *et al.*, 1976). Inflammatory findings were scarce in this study. An increase of secretion was suggested in exocrine cells, particularly chief cells and parietal cells. A large amount of *Vibrio cholerae* and cholera toxin was included in the regurgitated fluid from the intestine and it was possible that the exocrine glands of the gastric

Table 6 Findings of stomach and large intestine

No.	Age	Time after onset	Stomach			Large intestine			
			Vacuolization of parietal cell	Decrease of granules in chief cell	Desquamation of epithelium	Cell infiltration to propria	Goblet cell Accumuration	Goblet cell Secretion	Goblet cell Post-secretion
2	6	a few hs.	+	+	+	+	+++	+++	−
3	3	7	?	−	++	++	−	++	+++
4	63	8	+	+	−	−	+++	+	−
7	8	10	±	−	++	++	+++	++	−
9	4	12	−	−	++	++	++	+++	+
10	3	13	−	+	+	+	−	++	+
11	6 mons	14	+	+	++	+	−	++	−
12	65	24	±	−	+	+	+	+++	++
13	2	24	++	+	+	+	++	+	−
14	5	30	−	+	+	−	++	++	−
15	3	32	±	+	+	−	++	++	++
16	4	33	+	−	+	+	++	+	−
17	8	40	+	+	−	+	++	++	+
18	14	40	−	−	+	−	+++	++	−
19	1	41	−	−	−	+	+++	++	+
20	2	7 ds.	±	+	++	++	+++	++	+++
21	6 mons	3 ws.	±	+	−	+	++	++	−

mucosa were stimulated by cholera toxin.

In the large intestine, inflammatory reactions of the lamina propria and increase of goblet cells in the epithelial layer were observed. These findings were compatible with the reports by other investigators.

MESENTERIC LYMPHNODES

Macroscopic findings:

Examination of mesenteric lymphnodes was performed on 18 cases. Lymphnodes were palpable in all the cases and the swelling was remarkable especially in infant and child cases within 14 hours. Lymphnodes of index finger size were seen in heaps. The size was small in the cases of 7 days and 3 weeks. In the adult cases, the number as well as the size were small. The swelling of lymphnodes in the other regions was milder.

Histological findings:

The following three types were observed separately or in combination.

i) Swelling of lymph follicles and reaction of germinal centers (Photo. 31)
ii) Diffuse proliferation of lymphocytes (Photo. 32)
iii) Swelling and proliferation of reticulum cells.

There was no infiltration of neutrophils, eosiophils and plasma cells. Hyperplasia of lymph follicles with reaction of germinal centers was remarkable in the infant and child cases within 14 hours, mild in the cases over 24 hours, and not seen in the cases of 7 days and 3 weeks. Proliferation of lymphocytes filling the cords and sinusoids was severe except in one case of 3 weeks and adult cases. Swelling of reticulum cells and diffuse, scattered or sinusoidal proliferation were seen in half of the cases and were severe in three cases. The reaction in all adult cases was mild.

SPLEEN

Macroscopic findings:

Moderate swelling of the spleen weighing 225 g and 190 g was respectively noted in two of four adult cases. There was no definite swelling in the other two cases as well as in the infant cases.

Histological findings:

There were great differences between infant and child cases and adult cases but no differences by the lapse of time. In infant cases, lymph follicles increased in size and particularly the germinal centers showed sizeable swelling, reaction and a tendency of necrosis (Photo. 33). Larger germinal centers reached 600 μ and many cell debris showing destruction were seen (Photo. 34). The red pulps were abundant in blood content which was distributed in both sinusoids and cords but mostly in the former. Resolution of tissue was evident in seven cases. Most of cords showed distension. Inflammatoly cell infiltration was generally mild, but polymorphonuclear leukocytes, eosinophils, lymphocytes, plasma cells and reticulum cells were occasionally noted. The above changes were slightest in the adult cases.

Table 7 Microscopic findings of mesenteric lymphnode and spleen

	No.	Age	Time after onset	Mesenteric lymphnode: Reaction of follicle	Mesenteric lymphnode: Diffuse proliferation of lymphocyte	Mesenteric lymphnode: Reaction of reticulum cell	Spleen: Follicle: Swelling	Spleen: Follicle: Necrosis of germinal center	Spleen: Red pulp: Congestion	Spleen: Red pulp: Resolution
Infant and child	2	6	a few hs.	++	+++	+	++	++	+	+
	3	3	7	+++	++	−	+	+	−	+
	5	6	10				++	+	±	−
	7	8	10	+++	++	++	+	++	+	+
	9	4	12	+++	+++	+	++	++	+	−
	10	3	13	+	++	+	+	++	+	−
	11	6 mons	14	++	++	−	++	++	±	+
	13	2	24	++	+	+	++	++	+	−
	14	5	30	+	++	++	−	±	+	−
	15	3	32	+	++	−	++	++	−	+
	16	4	33	−	++	±	++	++	+	+
	17	8	40	+	++	+	±	+	++	−
	19	1	41	++	++	+	++	++	+	−
	20	2	7 ds.	−	+++	+	++	±	±	+
	21	6 mons	3 ws.	−	−	++	+	±	+	−
Adult	1	45	a few hs.				+	+	+	−
	4	63	8	−	−	±	−	±	−	−
	6	20	10	+	+	+	+	−	+	−
	12	65	24	−	−	±	−	±	±	−
	18	14	40	−	++	−	+	−	+	−

Comment on mesenteric lymphnodes and spleen:

Hyperplasia of lymphoid tissue in the intestinal wall has long been known. Hyperplasia of mesenteric lymphnodes was noted also by Norris (1973). Reactions of lymph follicles in the spleen were observed by Norris (1973) and Sun *et al.* (1971). Norris (1973) described that the size of mesenteric lymphnodes and lymph follicles in the spleen did not change.

It was clarified in this study that reactions of the spleen and mesenteric lymphnodes were more remarkable in infants and children like lymphoid tissue of the intestinal wall. The reactions of lymphoid tissues of the intestinal wall, mesenteric lymphnodes and the spleen, especially lymph follicles, showed similarity and were considered to have been developed by the same agent. It has been known that the germinal centers of lymph follicles react to a shock but are not swollen. The fact that only mesenteric lymphnodes were swollen among other lymphnodes may suggest that this reaction was enterogenic, i.e., the possibility that some stimulating substance was absorbed from or produced in the intestine.

LIVER

Macroscopic findings:

There was no manifest increase in weight nor swelling in the parenchyma of the liver. Acute congestion of moderate or severe degree was noted in 12 cases. The

consistency was somewhat decreased. Fatty metamorphosis frequent in the cases over 24 hours. The gall bladder was filled with a large amount of pale green bile with hypoviscosity in many cases.

Histological findings:

Severe degeneration and necrosis of liver cells were not observed. Fatty metamorphosis was observed in all cases (Photo. 35) except for case No. 4 and case No. 5 with cirrhosis. Table 8 shows the degree of metamorphosis, size of fat droplets and distribution in acini in corse of time. Since fatty liver in malnutrition was frequent, nutritional state at the autopsy was also described. Fatty metamorphosis was definitely severer in the cases over 24 hours as compared with the cases within 14 hours. Fat droplets were large in the cases of severe fatty metamorphosis (Photo. 36) and were fine in the cases of mild fatty metamorphosis (Photo. 37). The distribution in acini showed a tendency to shift from centroacinar one to diffuse one. In the subjects of this study, nutritional state was not always poor as shown in the Table and consequently there was no correlation between nutritional state and the degree of fatty metamorphosis.

Mobilization of Kuppfer's cells was seen in most cases (Photo. 38). Lymphocytes

Table 8 Fatty change of liver

No.	Age	Time after onset	Fatty change			Nutritional state
			Degree	Size of fat droplet	Distribution	
1	45	a few hs.	+	S	D	
2	6	a few hs.	+	S	C M	
3	3	7	++	S	C M	G
4	63	8	−			N
5	6	10	−			
6	20	10	+++	L	C	
7	8	10	+	S	C	N
8	2	11	+	L	D	G
9	4	12	++	M	D M	N
10	3	13	++	L	P	B
11	6 mons	14	++	S	P	N
12	65	24	++	S	D	N
13	2	24	+++	L	D	G
14	5	30	++	M	P C	B
15	3	32	+++	M	P M	G
16	4	33	+++	M	D	N
17	8	40	+++	L	D	G
18	14	40	+	S	C	G
19	1	41	+++	L	D	B
20	2	7 ds.	+++	L	P M	
21	6 mons	3 ws.	+++	L	D	B

S: small C: central G: good
M: medium M: mid zonal N: normal
L: large P: peripheral B: bad
D: diffuse

and neutrophils, though few in number, were seen in the sinuses in half of the cases. In the portal areas, mild round cell infiltration was seen, and dilatation of the branches of the portal veins was noted in 13 cases.

Comment on liver:

In the autopsy reports by Sun *et al.* (1971) and Norris (1973), fatty metamorphosis of the liver was observed in numerous cholera patients. They attributed the fatty metamorphosis to malnutrition and considered no relation to cholera infection. In the present study, the nutritional state of patients was not necessarily poor. Fatty metamorphosis was positively severe in the cases over 24 hours after the onset of cholera as compared with the cases within 14 hours. It is possible that some of the patients in this study had fatty metamorphosis before the onset of cholera. Nevertheless, it is certain that fatty metamorphosis of the liver was intensified by cholera infection, and it is suggested that cholera infection may possibly induce fatty metamorphosis of the liver.

As the possible mechanism, (1) direct effect of absorbed cholera toxin on the liver through the portal tracts, (2) ischemia and anoxia due to shock resulting from dehydration, and (3) acute nutritional deficiency resulting from dysfunction of the small intestine because of cholera, can be considered.

PANCREAS

Histological findings:

Inflammatory changes were not seen in either the interstitium or parenchyma. To examine the secretory condition of the pancreatic juice, zymogen granules in acinar cells that are enzymic component were studied. Zymogen granules were stained by Hematoxyline-eosin stain, PAS stain, Heidenhain's azocarmine stain and Launoy's method, but Heidenhain's azocarmine stain was the most effective.

In three cases dying within 8 hours after the onset, the secretion of zymogen granules was likely to be just completed and the supranuclear portion or the entire cytoplasm was foamy with a scanty amount of zymogen (Photo. 39). However, the quantity and distribution of zymogen were different by acinus or individual cell. Among six cases of 10–14 hours, zymogen granules were increased filling the cytoplasm in four cases (Photo. 40), and a moderate amount of zymogen granules together with somewhat large vacuoles at the base of the cytoplasm were observed in two other cases. A variety of quantity with a tendency of gradual decrease with time was seen in seven cases of 24–41 hours. Mild atrophy of the acinus was shown in one case of 40 hours (Photo. 41).

In two cases of 7 days and 3 weeks, zymogen granules were hardly recognized, the acinus was atrophic and the lumen of the acinus was dilated containing dense fluid material. Fibrosis was seen in the interacinar space (Photo. 42).

Comment on pancreas:

The results of this study suggest that the exocrine system of the pancreas might be stimulated in its function for production and secretion of digestive juice in cholera.

Table 9 Microscopical findings of pancreas

No.	Time after onset	Amount of zymogen granule	Atrophy of acinus	Others
2	a few hs.	±		foamy in apex
3	7	+		foamy in supranucleus
4	8	±		foamy in cytoplasm
6	10	++		
7	10	++		
8	11	++		
9	12	+		vacuole in base
10	13	+		vacuole in base
11	14	++		
13	24	+		
14	30	++		
15	32	±		
16	33	+		
17	40	±		
18	40	−	+	
19	41	+		
20	7 ds.	−	++	dilatation of lumen
21	3 ws.	−	++	dilatation of lumen, fibrosis

In the very early stage of the disease, microscopical examination of the acinar cell yields the feature which is usually found after the secretion of zymogen granules. In the second stage, 10–14 hours after the onset, the acinar cell is accumulated with the granules. The cases with long time course show the exhaustion of acinar cells.

Watanabe (1968) described in his paper entitled "The importance of pancreatic excretion from the standpoint of acute diarrheal diseases", that as for the mechanism of hypersecretion of digestive juice, taking cholera as an example, the following points are to be taken into consideration:

1) Cholera toxin contains a secretin-like substance stimulating secretion of pancreatic juice.

2) Cholera toxin directly stimulates the duodenal mucosa to release secretin.

3) Cholera toxin-induced inflammation of the duodenum itself leads to releasing secretin.

The data reviewed here are concerned with secretion of zymogen granules that are digestive enzymes. Greenough (1965) proposed the hypothesis that cholera is a syndrome of hypersecretion of secretin or secretin-like hormons, secondary to stimulation of the upper gut mucosa by cholera vibrios. Secretin causes the pancreas to secrete a large amount of fluid containing bicarbonate with little enzymic activity. Pancreozymin causes secretion of digestive enzymes. When they act coordinately, a large volume of enzyme-rich pancreatic juice is secreted from the pancreas. Besides, the pancreatic exocrine response is probably mediated by gastrin, glucagon and vasoactive intestinal peptide (VIP). It is interesting to note whether the level of

these hormones in blood is raised or not in the cholera patient (Morishita *et al.*, 1976). Fujita *et al.* (1975) found the release of serotonin and some polypeptide but did not find secretin nor pancreozymin from the rabbit duodenum by administration of cholera toxin into the loop. At least, in human cholera, the severest inflammatory condition is found in the duodenum, the mucosa of which shows edema and marked infiltration of inflammatory round cells, besides the structural changes such as fusing or atrophy of villi. This acute inflammation of the duodenum might be strong enough to release some hormones from the duodenum which stimulate the pancreas.

There might be the possibility that the natural cholera stimulates the exocrine cells of the digestive system including the stomach, intestines and pancreas to promote their secretion and production.

LUNG

Macroscopic findings:

Hyperemia was recognized in six cases but the other cases were all anemic. Mucous secretion in the trachea and bronchus was noted in one case within 14 hours and in seven cases over 24 hours.

Histological findings:

Bronchial changes and pulmonary changes probably due to aspiration of gastric content containing a large amount of *Vibrio cholerae* and cholera toxin were notable.

i) Bronchus

There was no such change as desquamation or erosion of the bronchial epithelium. Acute bronchitis was observed in no more than two cases among 17 cases not including three cases of chronic bronchitis. In some cases, the lumen of the bronchus was filled with fluidal content and desquamative epithelia. In half of the cases, goblet cells in the bronchial epithelium were increased in number, indicating increased secretion or accelerated production of mucus (Photo. 43).

ii) Aspiration pneumonia

Severe aspiration pneumonia was not recognized but inflammatory reaction seemingly due to aspiration was noted in 15 out of 20 cases. Infiltration of eosinophils and lymphocytes and proliferation of macrophages phagotizing brown pigments deriving from vomitus were seen in the intraalveolar or peribronchial region (Photo. 43). Infiltration of neutrophils was mostly mild. There was no appearance of foreign giant cells.

Comment on lung:

Repeated vomitings were noted in most cases. The possibility of inhalation is great especially in the state of cloudy consciousness. Dyspnea and cough were also noted in many cases.

Norris (1973) reported that pulmonary pathology was noted in 87 per cent and severe bronchopneumonia in 41 per cent. In the present study, reaction to aspiration was recognized in 75 per cent although there was no case of severe bronchopneumonia induced by cholera.

Increased secretion of goblet cells in the bronchial epithelium was noticed by Shozawa and Sekine (1974), who later suggested the possibility that the mucus leads to obstruction of airway and reapiratory distress.

HEART

Macroscopic findings:

Despite the prominent decrease of circulating blood by dehydration, the right atrium and the right ventricle were filled with blood and the wall was dilated. The blood was dark red and highly viscous because of severe dehydration.

Histological findings:

Myocarditis and necrotic foci of myocardium were not recognized. Vacuolation of perinuclear cytoplasm was noted in 13 cases (Photo. 44). Staining of the myocardium by Heidenhain's azocarmine stain was irregular in 15 cases (Photo. 45). This change showed no difference by time course or age. Fatty change disclosed by oil-red O stain for fat was minimal, being recognized only in two cases of 40 hours and 7 days.

Comment on heart:

The effects of cholera on hemodynamic, particularly hypovolemia and acidosis have been described in detail by Harvey *et al.* (1968). The central blood volume was large especially in the right atrium and the right ventricle that showed dilatation also in the present study.

Anitschkow myocytes that were found by Goldstein *et al.* (1966) upon venous injection of *Vibrio cholerae* to guinea pig and by Sun *et al.* (1971) in their autopsy cases, were not observed on this histological examination. Focal myocarditis described by Norris (1973) was not observed either.

In the present study, exhaustion of the myocardium was speculated in view of the irregularity in staining by Heidenhain's azocarmine stain and the perinuclear vacuolar change.

Fatty change of myocytes observed in the liver and kidney was noted only in two cases and it was quite minimal.

KIDNEY

Macroscopic findings:

The kidney showed no increase of weight nor swelling of parenchyma.

Histological findings:

There was neither interstitial edema nor round cell infiltration. The cortex showed congestion in a few cases but it was obviously anemic in some other cases. The medulla showed congestion in half of the cases. The glomeruli were not remarkable. The tubular epithelium was tall with narrow lumen and scant content.

In eight of 11 cases dying within 14 hours after the onset, severe hyaline droplet

Table 10 Microscopic findings of kidney

No.	Age	Time after onset	Hyaline droplet formation	Fatty change	Vacuolar change
1	45	a few hs.	+		−
2	6	a few hs.	±	±	−
3	3	7	++	−	−
4	63	8	++	±	−
5	6	10	+	±	−
6	20	10	++		−
7	8	10	++	−	−
8	2	11	++		−
9	4	12	++	−	−
10	3	13	++	+	−
11	6 mons	14	++	+	−
12	65	24	++	+	−
13	2	24	±	++	−
14	5	30	+	+	−
15	3	32	−	+++	−
16	4	33	±	+++	−
17	8	40	−	+++	±
18	14	40	++	±	−
19	1	41	+	+++	−
20	2	7 ds.	−	+++	++
21	6 mons	3 ws.	±	+	++

formation focal to the neck portion of proximal tubules was observed (Photo. 46). Hyaline droplets were also present at the urinary pole of Bowman's capsule or in the proximal convoluted tubules in some cases. Hyaline droplet formation of the entire proximal tubules was seen in a few cases. Among the cases over 24 hours, only two cases showed remarkable hyaline droplet formation.

Fatty change of the tubular epithelium was seen mostly in the cases over 24 hours (Photo. 47), and the change was severe in five cases. The change extended to the entire tubules in severe cases but localized to the proximal tubules in mild cases. The fatty change of the kidney was comparable with that of the liver in its degree in each patient.

Coarse vacuolation of the tubular epithelium as noted in hypokalemia was seen in two cases of 7 days and 3 weeks (Photo. 48).

Patchy necrosis of the tubular epithelium was recognized only in one case of 40 hours (Photo. 49). Cloudy swelling or degeneration was severe in three cases and mild in the other cases.

Comment on kidney:

The renal failure as a complication of cholera has been a matter of great concern.

As a cause of this disturbance, the followings have been implicated; acute fall in blood pressure (Basu, 1961), hemoconcentration, acidosis, anoxia (De *et al.*, 1954) and hypokalemia (Benyajati *et al.*, 1960). Histological features in the cholera kidney have been reported as necrosis and fatty change in the cortical tubules following cortical ischemia and medullary congestion (De *et al.*, 1954), and hypokalemic vacuolation (Benyajati *et al.*, 1960).

Many observers accept recently the concept that hyalin-droplet formation is due to reabsorption of the protein in the glomerular filtrate by the epithelium in the proximal tubules. As total blood volume is decreased and the plasma protein is of high concentration in the patient before establishment of rehydration, the volume of the glomerular filtrate might be decreased and its concentration of protein might be high. It is interesting that hyaline droplets were seen in many of our cases especially in the cases dying within 14 hours and were strictly limited in and near the neck portion of the tubules, probably because of decrease of volume and high concentration of protein in the glomerular filtrate. Droplets in the cases over 24 hours disappeared, which might be digested in the cells within a short time after rehydration.

Fatty change should be progressive in the early stage of cholera. It is noticed that fatty change corresponded between the kidney and liver. This might indicate the presence of some simultanously acting factor in the blood causing this change to the kidney and liver.

Hypokalemic nephropathy characterized by coarse vaculoation of tubular epithelium was present in long standing cases, 7 days and 3 weeks. This is consistent with the observation that the vacuolation takes about a week to develop in man.

In our cases of early stage of cholera patients dying before 41 hours, tubular degeneration and necrosis were mild as some observers have reported.

ADRENALS

Histological findings:

Mild hyperemia was present in some cases. Inflammatory cell infiltration, hemorrhage and focal necrosis of the cortical cells were not observed. Functional changes due to hypovolemic shock were seen in the fascicular zone. Clear like cells decreased and compact like cells (Photo. 50) increased with the course of time especially in cases dying after 24 hours. Oil red O stain was performed on 10 cases and marked decrease of lipid was recognized in two cases (Table 11).

Comment on adrenals:

Rich (1944) observed tubular formation involving inflammatory reactions of the solid cords of the cells of the fascicular zone in diphtheria and other severe infections, but no such changes were seen in the present study. De *et al.* (1955) reported that they could not find any remarkable change by ordianary staining method in examination of adrenals of cholera patients, and pointed out evidence of various degrees of depletion of cortical lipid. They speculated that this might be the active role of the cortex in response to the stress by dehydration of cholera. In our study also, histological abnormalities were not observed in the cortex, and changes due to

Table 11 Microscopic findings of lung, heart and adrenal gland

No.	Age	Time after onset	Lung			Heart		Adrenal gland	
			Changes by aspiration	Peribronchitis	Hypersecretion of goblet cell	Vacuolar change	Irregularity in staining	Predominant cell in cortex	Depletion of lipid (oil red O)
1	45	a few hs.	−	−	−	+	+	clear, compact	
2	6	a few hs.	+	−	+	+	+		++
3	3	7	+	−	−	++	+		−
4	63	8	−	−	−	−	+	clear, compact	
5	6	10	+	−	−	−	+		
6	20	10	−	chronic	++	+	+	clear	
7	8	10	+	+	+++	+	++	compact	
8	2	11	+	−	+	++	+		
9	4	12	+	chronic	+	+	−	compact	++
10	3	13	+	+	−	++	+	clear	−
11	6 mons	14	+	−	+	−	+	normal	−
12	65	24				−	−	clear, compact	
13	2	24	+	chronic	−	+	−	clear	−
14	5	30	+	−	++	+	+	compact	+
15	3	32	+	−	+++	−	−	clear	+
16	4	33	+	−	+++	−	−	compact	±
17	8	40	+	−	−	−	++	compact	
18	14	40	+	+	+++	+	++	compact	
19	1	41	−	−	−	++	+	compact, normal	+
20	2	7 ds.	+	−	−	−	−	compact, normal	
21	6 mons	3 ws.	−	−	−	++	+	clear	

the stress which seemed physiological were seen in the fascicular zone.

BRAIN

Macroscopic findings:

Study of the brain was performed on five cases. The brain showed swelling (Table 12). Particularly in two cases of age 2, the swelling of the brain was remarkable, weighing 1,300 g, and showing tonsilar herniation.

Histological findings:

There were observed retraction of the nerve cell body and pericellular edema (Photo. 51), both of which were considered ischemic changes.

Comment on brain:

The cerebro-neural symptoms observed in this series were cramps in three infant and child cases and severe disturbance of consciousness in many cases. These

symptoms were seemed due to circulatory disturbance of the brain caused by severe hypovolemic shock. Cerebral swelling and histological abnormalities such as edema and retraction of nerve cells seem to have resulted from ischemia and anoxia. It is estimated that these changes should be related to the cause of death.

Table 12 Findings of brain

No.	Age	Weight of brain (g)	Pressure cone	Pericellular edema Cortex	Basal ganglia
8	2	1,300	+	±	±
10	3	1,130	−	−	±
13	2	1,300	+	+	+
15	3	1,150	−	+	±
17	8	1,270	−	+	+

Conclusions

i) Effects of cholera on organs in the body

Unlike *Shigella* (Takeuchi *et al.*, 1965), *Vibrio choleae* does not penetrate through the intestinal epithelium nor does enter the blood via the lamina propria as seen in *Salmonella* (Takeuchi and Sprinz, 1967; Takeuchi, 1971). There have been reports that in rabbit cholera toxin was not found in the lamina propria or blood vessel even when it was found in the supranuclear portion of the epithelium, and that in mouse cholera toxin adhering the surface of microvilli would not penetrate through the epithelium (Peterson *et al.*, 1972).

The evidence that hyperglycemia and the increased levels of serum alkaline phosphatase (SAL–P) and glutamic-oxalacetic transaminase (SGOT) resulting from intravenous administration of cholera toxin in dog is not recognized in cholera patients (Pierce *et al.*, 1972) supports that cholera toxin is hardly absorbed. However, a minimal amount may be absorbed since the level of antitoxic antibodies in the serum of the patients increases (Pierce *et al.*, 1970). In the experiment of forming two loops in the intestine of the rabbit and injecting crude cholera toxin in one loop (Serebro *et al.*, 1968), observed accumulation of fluid in the other adjacent loop in which the mucosa had no direct contact with toxin, Vaughan-William and Dohadwalla (1969) reported that injection of cholera toxin and *Vibrio cholerae* in the intestine of one rabbit by cross circulation experiments on paired infant rabbits resulted in accumulation of fluid and diarrhea in the intestine of the other rabbit. These phenomena suggest that some diarrhogenic substance is present in the blood affected by cholera toxin.

Fujita *et al.* (1975) found that cholera toxin administered into the rabbit duodenum caused severe degranulation of enterochromaffin cells especially of those cells secreting serotonin and some peptide (Motilin?), and pointed out the possibility that these are the circulating messengers of diarrhogenic action of cholera toxin.

cAMP was found by Sutherland and Rall (1958), Field *et al.* (1968) and Field (1971) found that cAMP, theophylline and cholera toxin demonstrate the same

mechanism in causing transport of fluid (Na^+ and Cl^-) in the intestinal mucosa. Subsequent studies (Chen *et al.*, 1971; Kimberg *et al.*, 1971) proved that cAMP and adenyl cyclase are diarrhogenic factors in cholera.

In addition to diarrhogenic action, cholera toxin is known to have various effects such as increased skin capillary permeability, enhancement of lipolysis by rat epididymal fat cells (Vaughan *et al.*, 1970), increase of adenyl cyclase activity in mouse liver cells (Gorman and Bitensky, 1972), and stimulation of glycogenolysis in human platelets (Zieve *et al.*, 1971) and rat liver cells. Morishita (1977) has observed increased level of cAMP in the blood of cholera patients.

If cholera is an event limited only to the intestinal lumen and its adjacent mucous epithelium with little or no absorption of cholera toxin, the changes in the body by cholera except for gastrointestinal changes and pulmonary changes due to aspiration must be secondary to hypovolemic shock, acidosis and hypokalemia resulting from dehydration.

In this study, some findings that cannot be explained only by dehydration resulting from diarrhea were found in some organs. First, these changes are hyperplasia of the mesenteric lymphnodes and inflammatory reactions of the spleen. Hyperplasia and reaction of the lymphoid tissues in the intestinal wall are understandable since these tissue are located where stimuli are directly received. However, the changes of the mesenteric lymphnodes and the spleen suggest the presence in the body of some stimulating substance deriving from the intestine.

Secondly, the changes are seen in the liver. Fatty metamorphosis of liver cells is hardly explained as a change due to dehydration. The liver is an organ easily influenced by the intestine through the portal vein. Pierce *et al.* (1972) observed hyperglycemia and increased levels of SGOT and SAL–P after parenteral administration of cholera toxin. The change of glycogen is related to cAMP. SGOT and SAL–P are also considered to derive from the liver.

The third changes are fatty change of renal cells, which is severe despite the mild change of the tubular epithelium due to circulation disturbance. The fourth changes are the increased production and secretion of zymogen granules in the pancreas. Consequently, factors deriving from the intestine seem to be active in various parts of the body. Sun *et al.* (1971) noted Anitschkow myocytes in the myocardium of cholera patients and damage to endothelial cells of the small blood vessel, although these changes were not observed in our series, and he attributed these changes to possible evidence of the toxicity of cholera toxin. This may also provide an endorsement.

ii) Cause of death in cholera patients

In comparative studies of autopsy findings between cholera patients dying before and after 1960, Norris (1973) states that over 50 per cent of patients (Cash *et al.*, 1973) dying of cholera succumbed of renal failure before 1960 but the frequency of renal lesions decreased to 5 per cent after 1960, and attributes this improvement to transfusion.

In the present study, the renal lesions were so mild as to be the possible cause of death. This may be due to the fact that most of the patients in this study died within 2 days after the onset before the appearance of severe renal lesions and they were provided with transfusion.

Dying of dehydration may be explained morphologically by cardiac failure due to exhaustion of myocardium and by herniation of tonsils due to swelling of the brain.

Respiratory disturbance due to aspiration of gastric content may also be considered as the cause of death in some cases.

Acknowledgements

The author wishes to express his grateful thanks to Prof. Issei Nishimori, Department of Pathology, Atomic Disease Institute, Nagasaki University, for helpful suggestions and continous encouragement, to the late Prof. Toyosuke Watanabe, Department of Pathology, Institute for Tropical Medicine, Nagasaki University, for guidance in this study and preparing autopsy materials, to Prof. Takeshi Schozawa, Department of Pathology, Akita University, for valuable advices, to Dr. Kazumine Kobari, the President of Hamamatsu Medical Center, for his kind advices and arrangement the autopsy in Manila, to Prof. Tatsuro Naito and Assis. Prof. Masaaki Iwanaga, Department of Bacteriology, Institute for Tropical Medicine, for the opportunity in presenting this paper, to Prof. Hideo Tsuchiyama, Department of Pathology, Nagasaki University, for kind guidance in studying about the adrenals in this study, to Dr. Masao Nakatomi, Department of Internal Medicine, Nagasaki University, for assistance in the autopsy in Manila, to Dr. Reyes, the Chief Doctor of Pathology, San Lazaro Hospital, and Dr. Uylangco, the President of San Lasaro Hospital, for permission to do the autopsy at their hospital, to Miss Toshiko Fukushima and Miss Yukie Kunita, for their excellent technical assistance in tissue specimen preparation.

This study was supported by U. S.-Japan Cooperative Medical Science Program, Cholera Panel.

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コレラ初期変化の病理学的研究

——21剖検例をもとに——

関　根　一　郎

1968-1970年マニラ市サン・ラザロ病院で死亡，剖検された21例のコレラ患者の病理形態学的観察を行った。コレラは発症後短時間で治癒または死亡する疾患であるが，特に材料を時間経過にもとづいて観察することにより，コレラ特有の病像のいくつかを浮揚せしめた。また形態的変化をもとにコレラの病態生理についても言及した。

材料（21症例）は次の特徴をもつ。1）7日・3週の2例を除き，発生後41時間以内の短時間で死亡している。2）小児が多く，16/21を占める。3）死後変化を阻止するため死後短時間で剖検され，20/21は2時間以内であった。

結論は次の通りである。

1）小腸粘膜異常は，炎症性反応とそれに続発する絨毛萎縮である。この萎縮はコレラ感染による炎症性刺激で，腺窩に細胞分裂亢進が起こったことが大きな役割を果たしていると考えられた。

2）外分泌細胞亢進がみられた。小腸・大腸・気管支の杯細胞，小腸パネート細胞の分泌亢進が示された。膵チモーゲン顆粒・胃底腺・胆汁の分泌亢進も窺われた。

3）コレラ毒素又は何らかの物質が体内へ侵入する可能性を示した。これは，a）腸間膜リンパ節及び脾のリンパ濾胞の反応，b）肝・腎の進行性脂肪変性，c）膵外分泌亢進，等により推察された。

4）コレラの死因として，脱水にもとづく心不全，脳浮腫が挙げられる。誤飲による呼吸障害も考慮されるべきである。脱水による腎不全は初期の適当な輸液により防ぎ得ると思われた。

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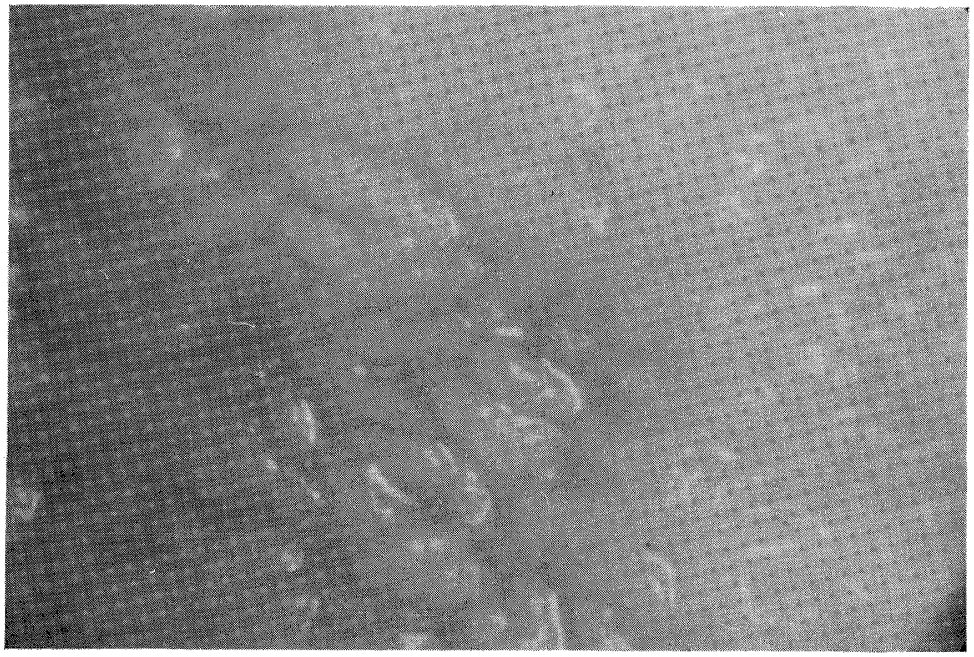

Photo. 1 Dissecting microscopic findings of the upper jejunum of No. 4., 63-year-old male of 8 hours. Almost villi are leaf like and many of them form ridges.

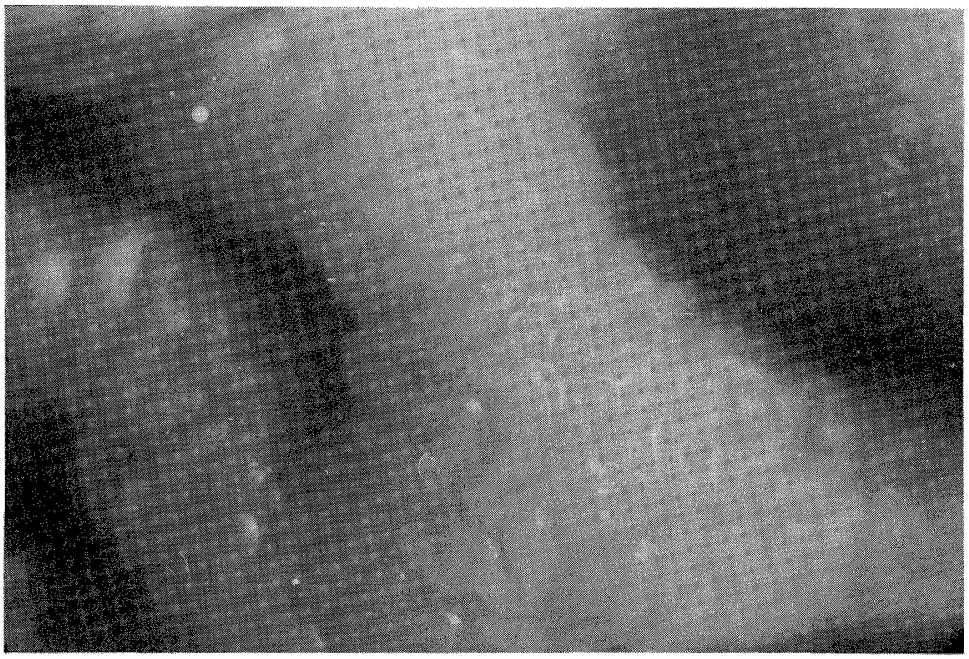

Photo. 2 Dissecting microscopic findings of the upper jejunum of No. 15., 3-year-old child of 32 hours. Convolutions are predominant features, which resemble a cerebral hemisphere.

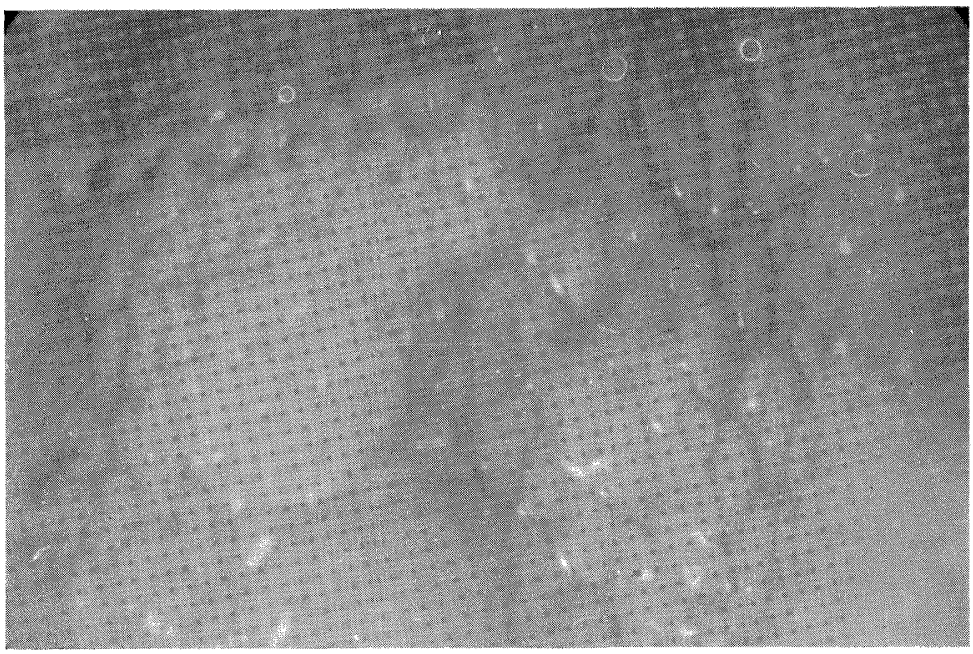

Photo. 3 Dissecting microscopic findings of the lower jejunum of No. 6., 20-year-old female of 10 hours. Each villus is separated with tongue shape essentially. But the view of the mucosa appears cobblestone-like because of blunting of the villi.

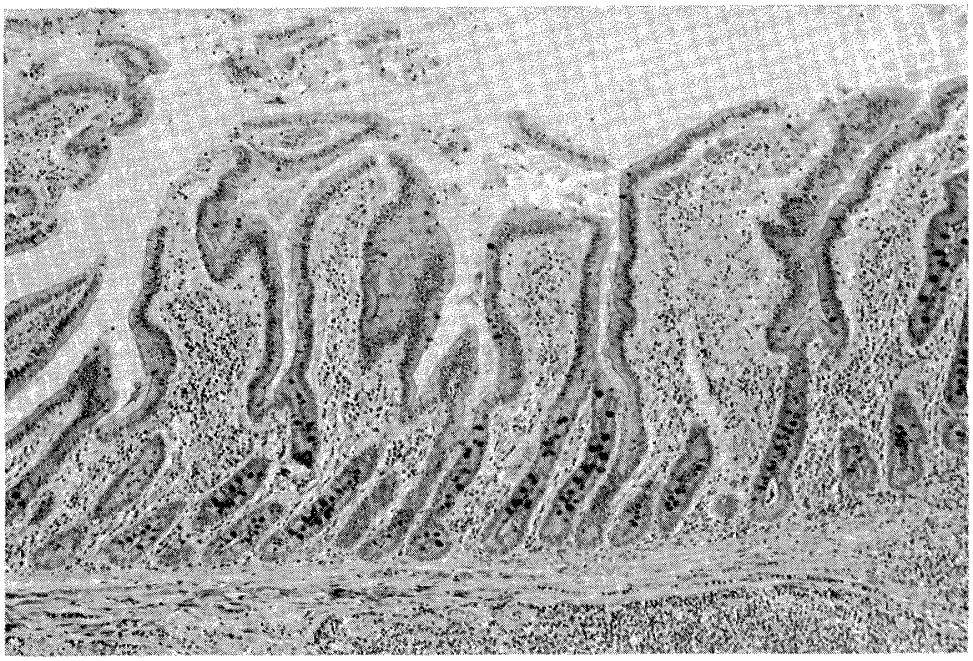

Photo. 4 Jejunum in No. 3., 3-year-old child of 7 hours.
The essential architecture of the villi is similar to that of the normal. Each villus is separated without fusions. The goblet cells are diminished in the free surface of the villi because of completion of secretion. The lamina propria is expanded by inflammatory cell infiltration. Villus to crypt ratio is about 1.5.
Alcian blue-PAS-Masson's trichrome. ×90.

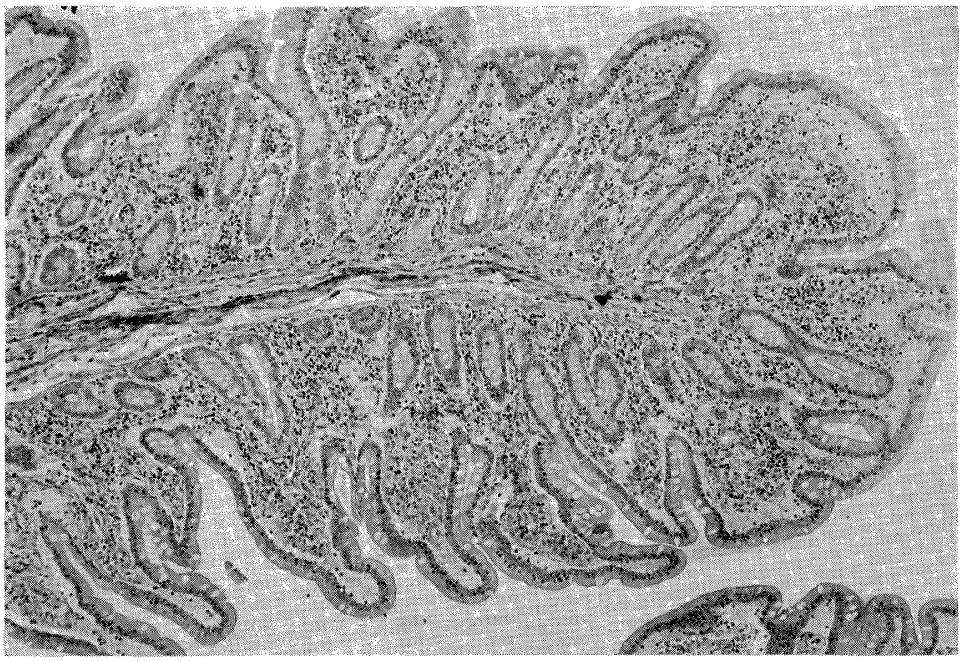

Photo. 5 Jejunum in No. 19., 1-year-old child of 41 hours.
The villi are atrophic. In the tip of the circular fold, the villi show fusions with the lamina propria. The crypts are elongated. Villus to crypt ratio is about 1.0 or less.
Heidenhain's azocarmine. ×90.

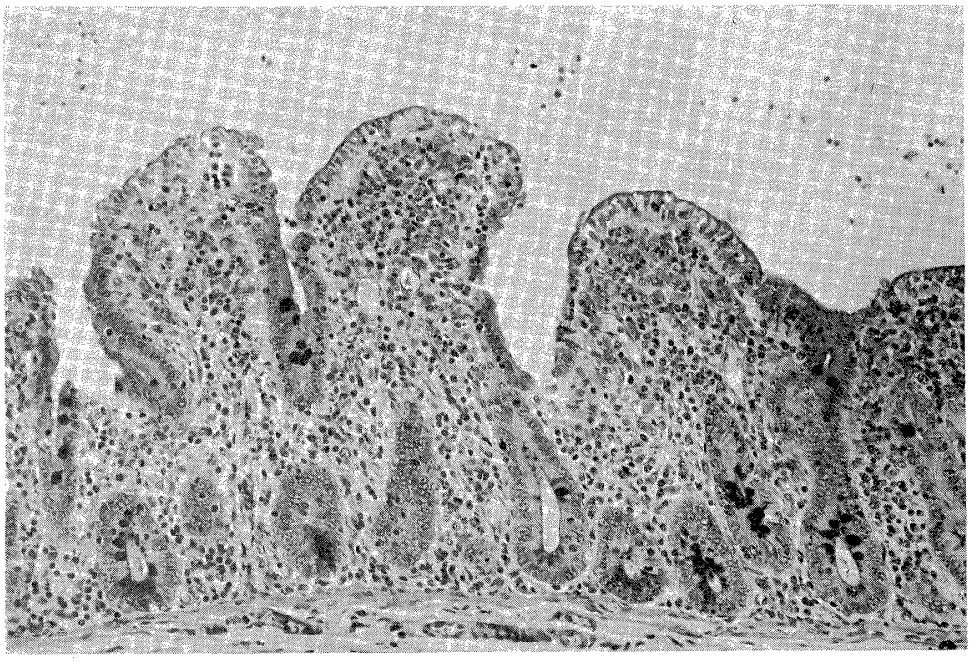

Photo. 6 Jejunum in No. 11., 6-month-old infant of 14 hours.
The villi are short and bulbous (blunt). The surfaced epithelium is degenerated. The lamina propria shows hypercellularity by inflammation.
Alcian blue-PAS-Masson's trichrome. ×180.

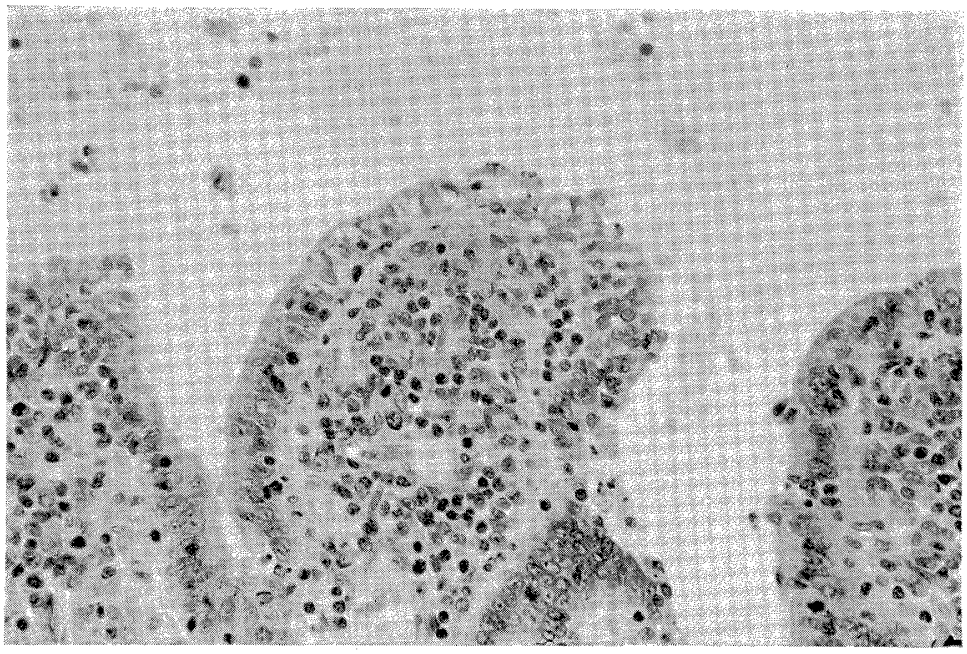

Photo. 7 Higher magnification of Photo. 6. Surfaced epitheli especially in the tip of the villus show intracytoplasmic vacuolation. Inflammatory cells migrate into the epithelial cell. H. & E. ×360.

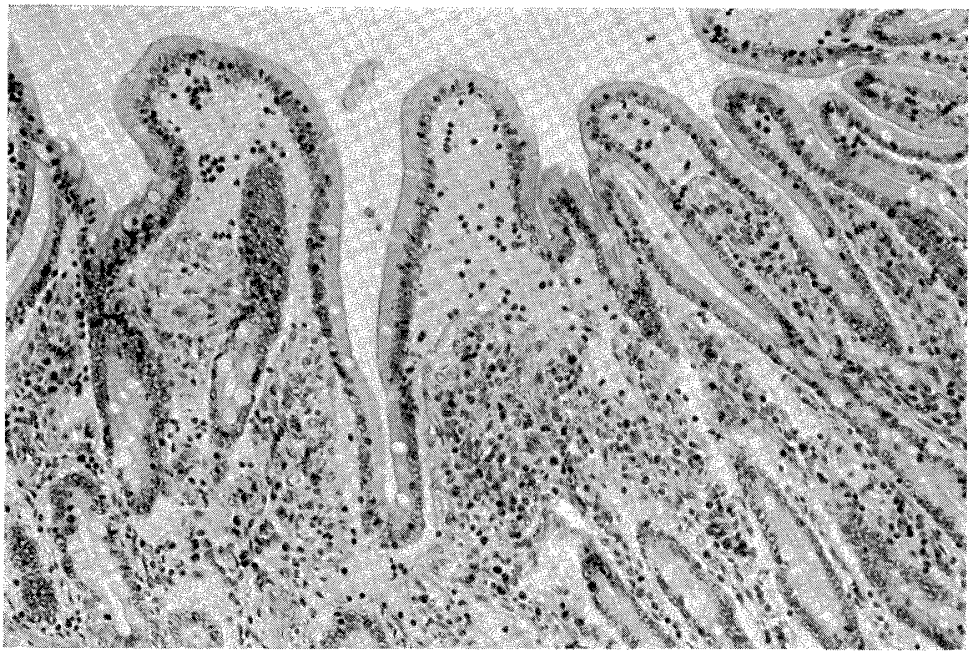

Photo. 8 Jejunum in No. 19., 1-year-old child of 41 hours.
Note tissue slits in the subepithelial region which contain fluidal material and inflammatory round cells.
Heidenhain's azocarmine. ×90.

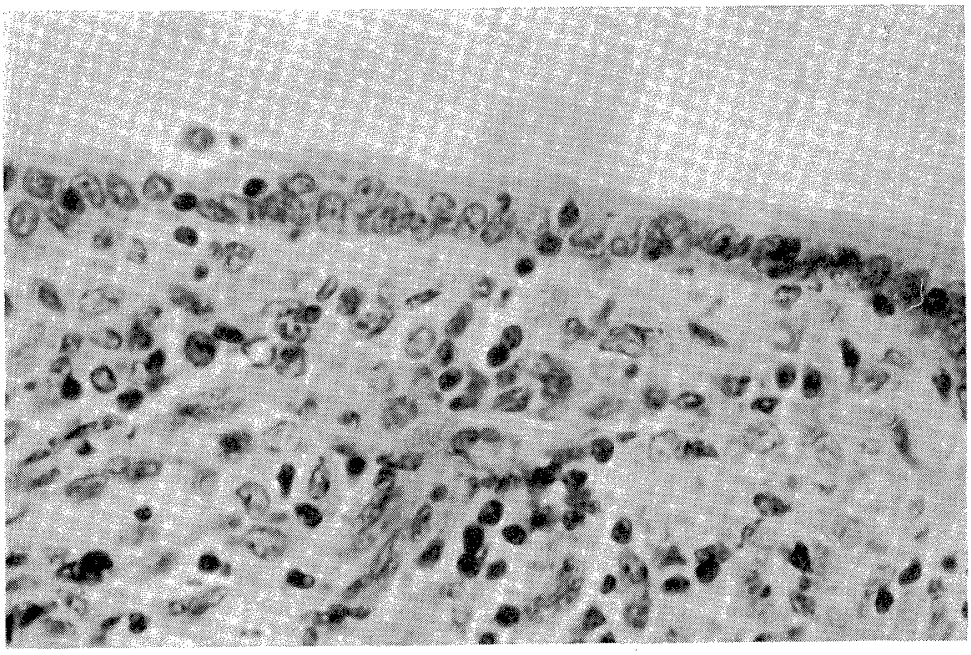

Photo. 9 Jejunum in No. 13., 2-year-old child of 12 hours.
Note cuboidal change of the epithelium. The brush border is preserved. The lamina propria shows hypercellularity.
H. & E. ×720.

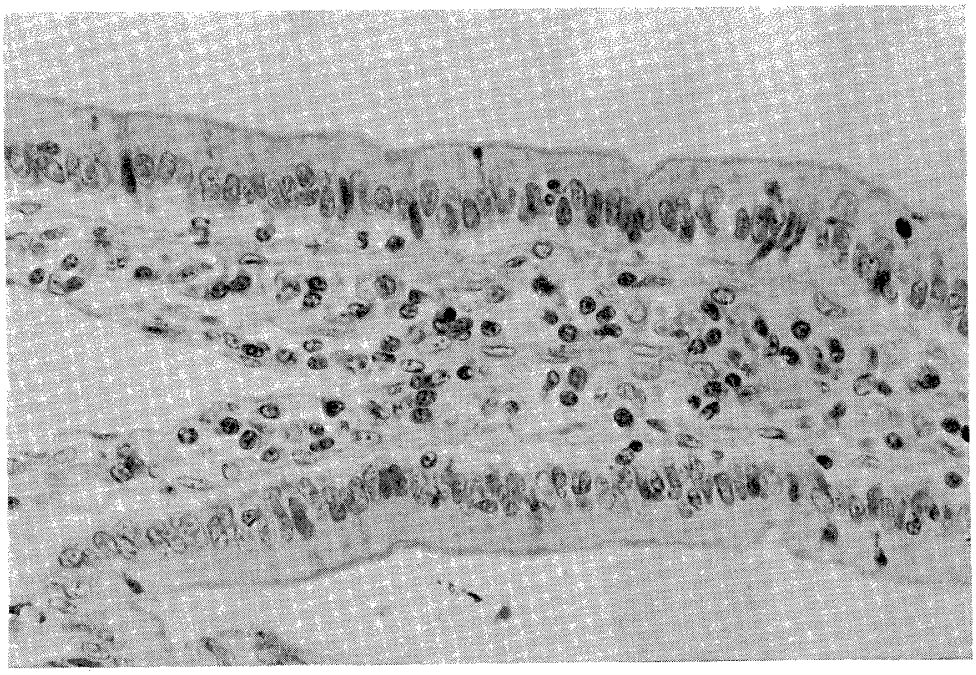

Photo. 10 Jejunum in No. 3., 3-year-old child of 7 hours. Note the tall columnar epithelium of the villus. The brush border is preserved. Goblet cells are diminished in size because of completion of secretion. The central lacteal is dilated.
Alcian blue-PAS- Masson's trichrome. ×480.

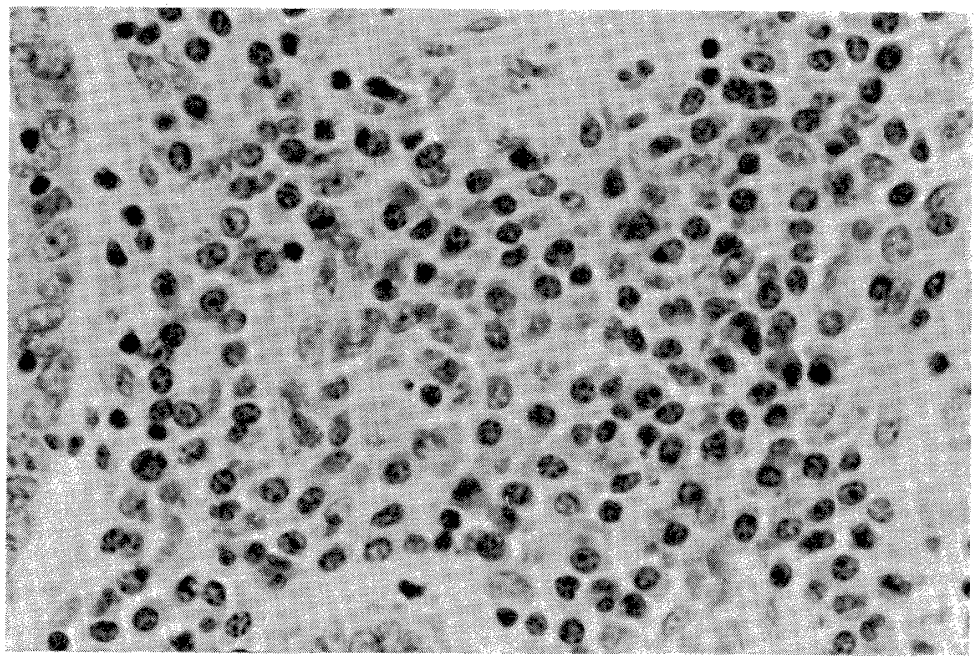

Photo. 11 Jejunum in No. 7., 8-year-old child of 10 hours.
Note extensive infiltration of plasma cells to the lamina propria.
H. & E. ×720.

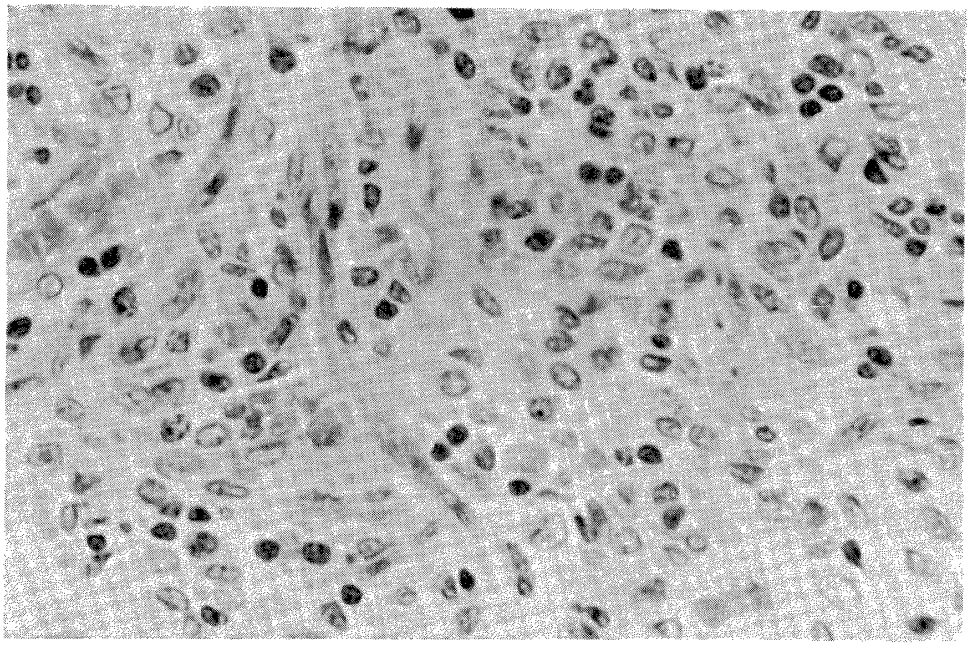

Photo. 12 Jejunum in No. 16., 4-year-old child of 33 hours.
Note proliferation of histiocytes in the lamina propria.
H. & E. ×720.

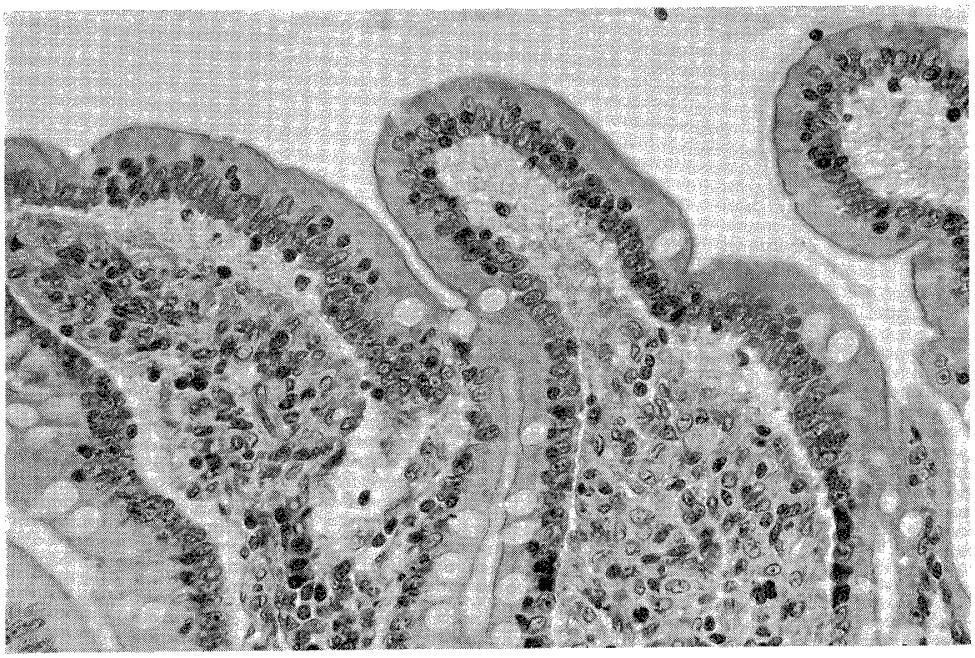

Photo. 13 Jejunum in No. 19., 1-year-old child of 41 hours.
Note fusion involving the surfaced epithelium of the two villi. The brush border is preserved. The size of goblet is increased.
Heidenhain's azocarmin. ×360.

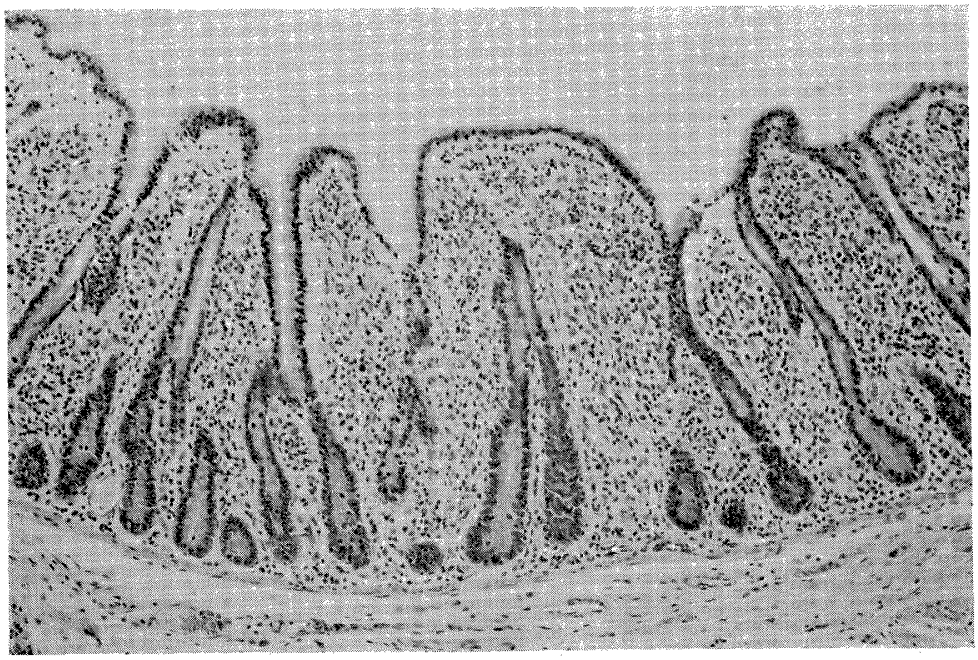

Photo. 14 Jejunum in No. 13., 2-year-old child of 24 hours.
Note fusion involving the lamina propria. Also epithelial fusions are seen. Villus to crypt ratio is 0.8 to 1.0. The surfaced epithelium is cuboidal. The hypercellularity is moderate in the lamina propria.
H. & E. ×120.

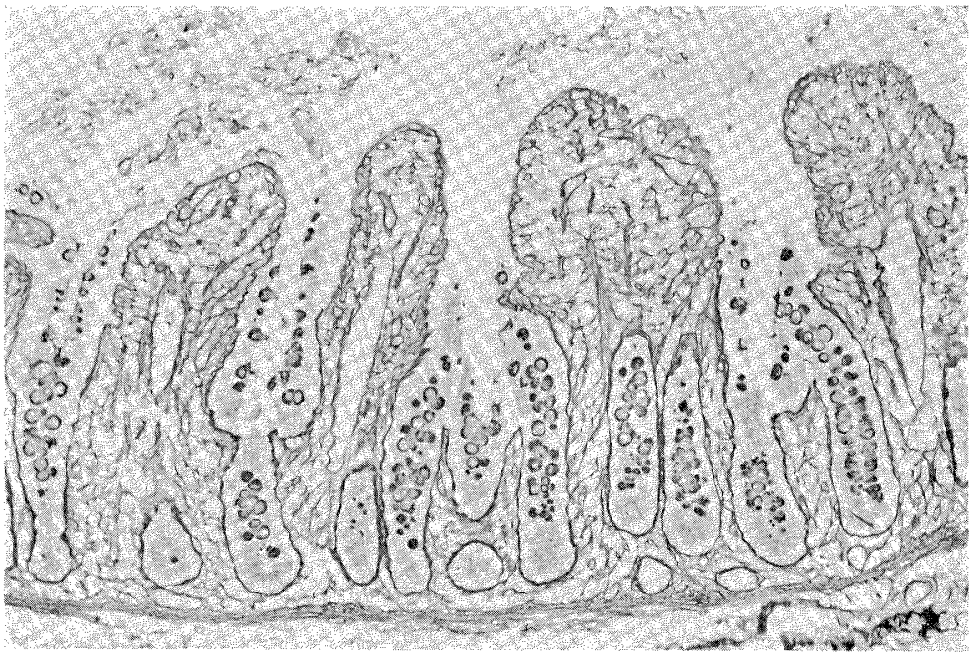

Photo. 15 Jejunum in No. 7., 8-year-old child of 10 hours.
The essential architecture of the reticulum fibers of the villus is separated but the lamina propria is broadened by inflammatory cell infiltration.
Silver impregnation for reticulum. ×180.

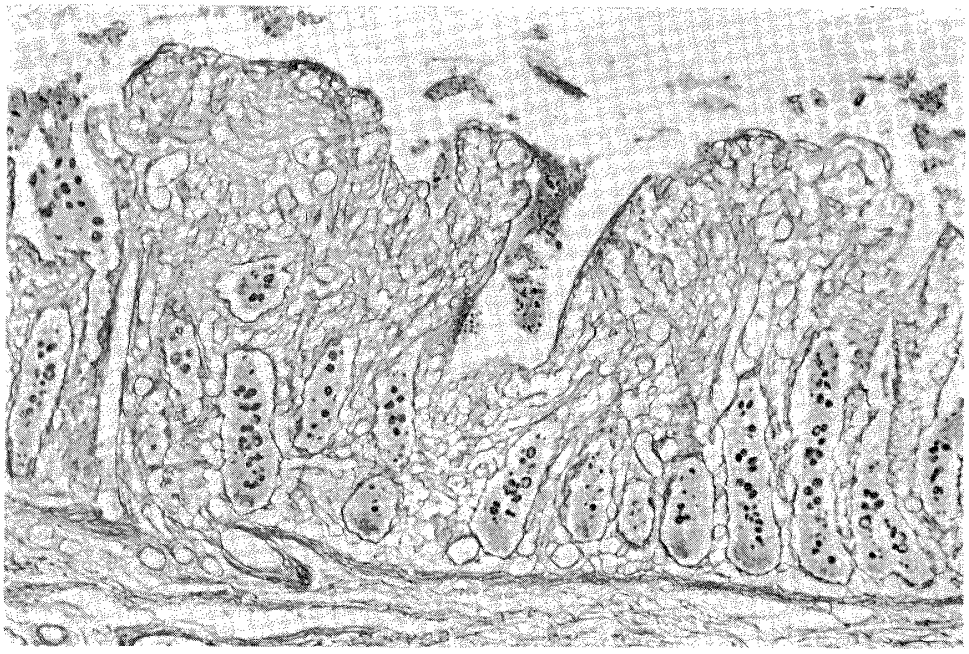

Photo. 16 Jejunum in No. 17., 8-year-old child of 40 hours.
Villi are fused with the lamina propria. The reticulum fibers show dence proliferation.
Silver impregnation for reticulum. ×180.

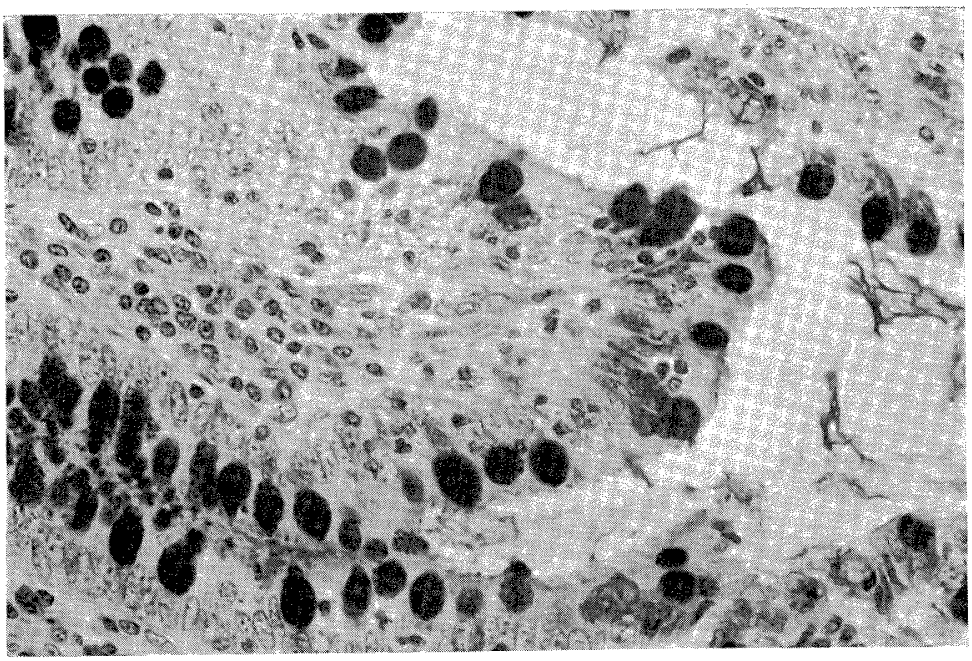

Photo. 17 Jejunum in No. 9., 4-year-old child of 12 hours.
The amount of the mucus in the goblet cells is prominently increased. The secretion already begins.
Alcian blue-PAS-Masson's trichrome. ×480.

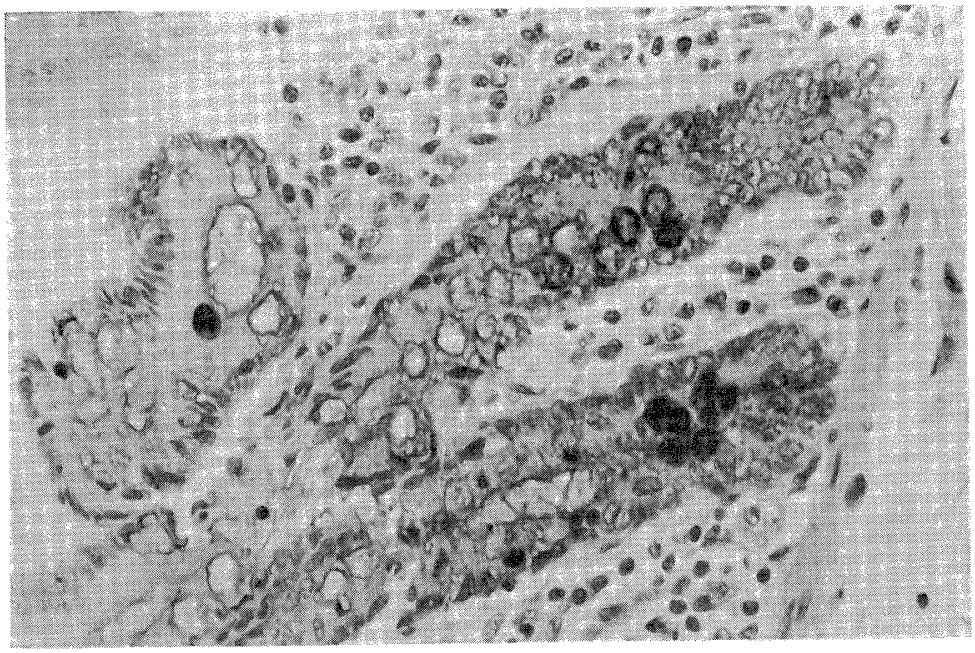

Photo. 18 Jejunum in No. 19., 1-year-old child of 41 hours.
The cytoplasm of the goblet cells is pale and sparse, which suggests the stage near the completion of exretion.
Alcian blue-PAS-Masson's trichrome. ×720.

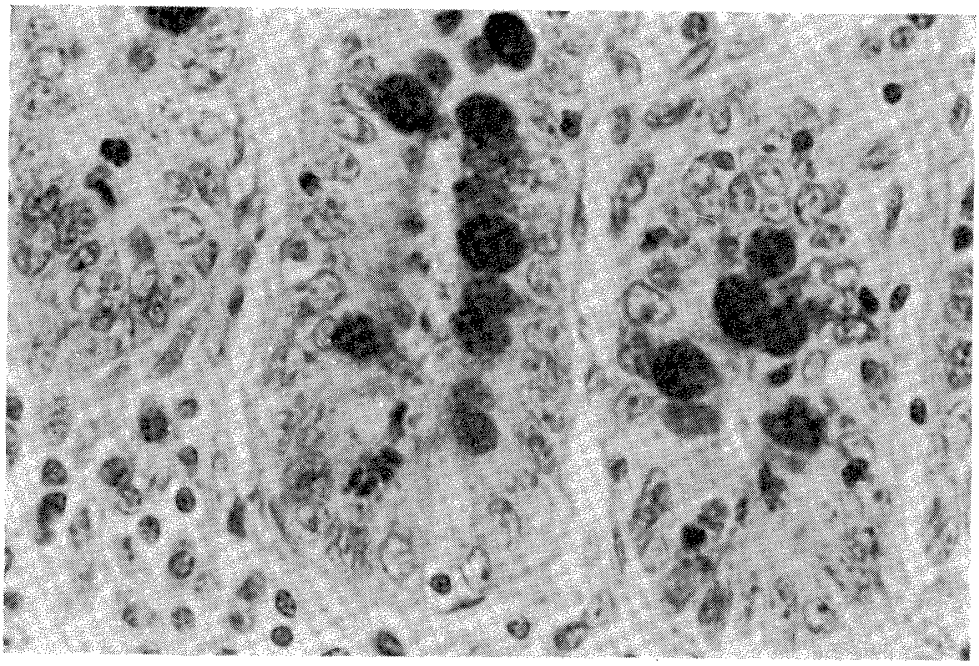

Photo. 19 Ileum in No. 7., 8-year-old child of 10 hours.
The apical portions of Paneth cell are pale and foamy likely to be completion of excretion of granules. Conglomerated granules are seen in some Paneth cells.
Alcian blue-PAS-Masson's trichrome. ×720.

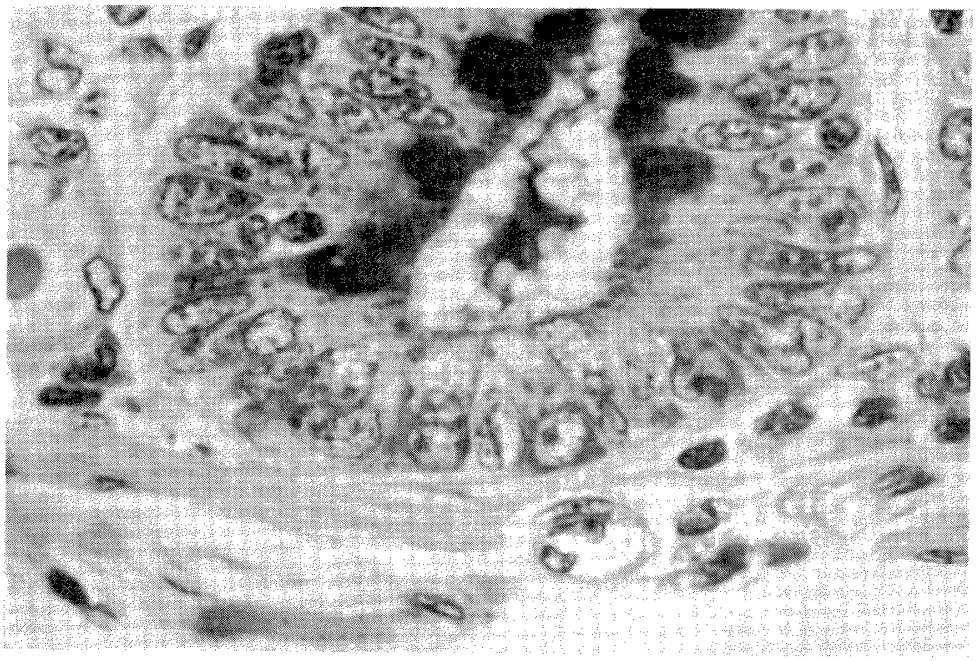

Photo. 20 Ileum in No. 9., 4-year-old child of 12 hours.
The apical portion of Paneth cell shows the foamy trace of excretory granules.
Alcian blue-PAS-Masson's trichrome. ×900.

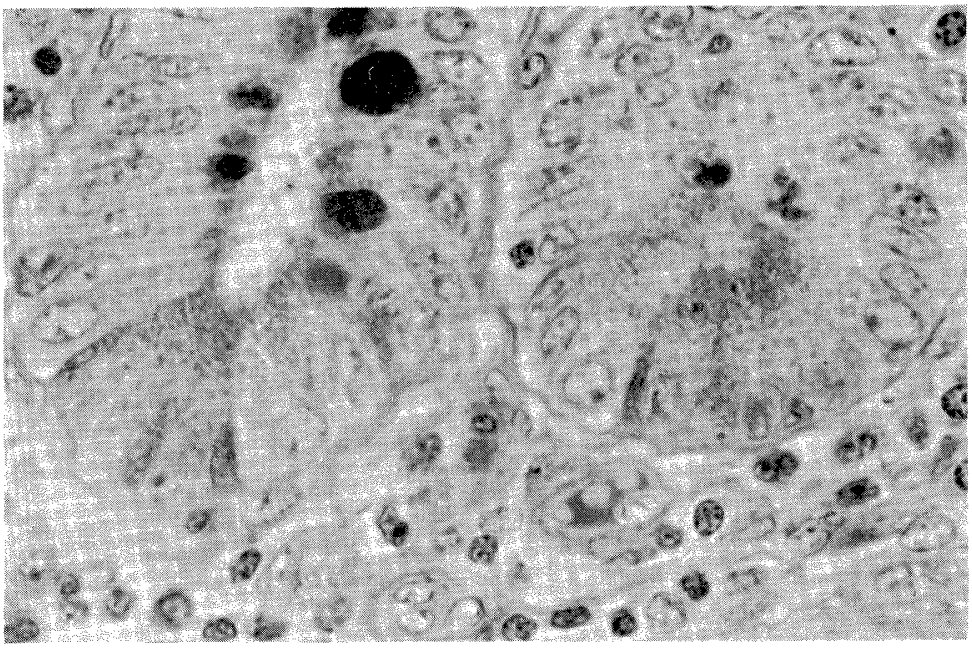

Photo. 21 Ileum in No. 7., 8-year-old child of 10 hours.
The apical portion of Paneth cells contains fine light green stained granules which are suggested to be immature granules. These granules are being secreated.
Alcian blue-PAS-Masson's trichrome. ×900.

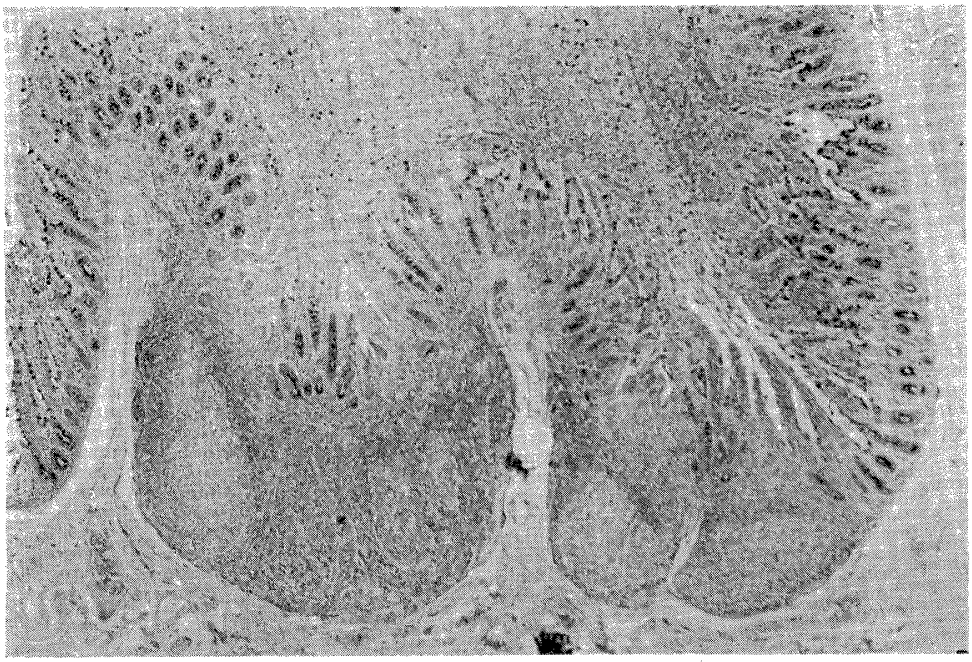

Photo. 22 Ileum in No. 9., 4-year-old child of 12 hours.
Lymph follicles in the mucosa are hyperplastic. Desquamation of epithelium is severe.
Alcian blue-PAS-Masson's trichrome. ×48.

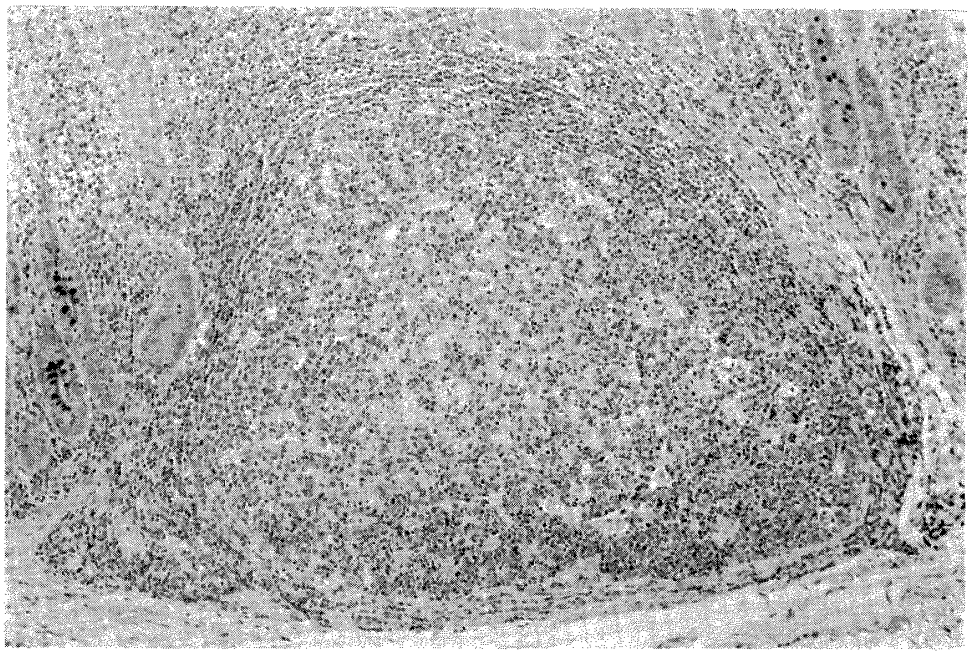

Photo. 23 Ileum in No. 9., the same case as Photo. 22.
Note reaction of the germinal center of hyperplastic lymph follicle, which shows cell debris due to necrobiosis of the tissue.
H. & E. ×120.

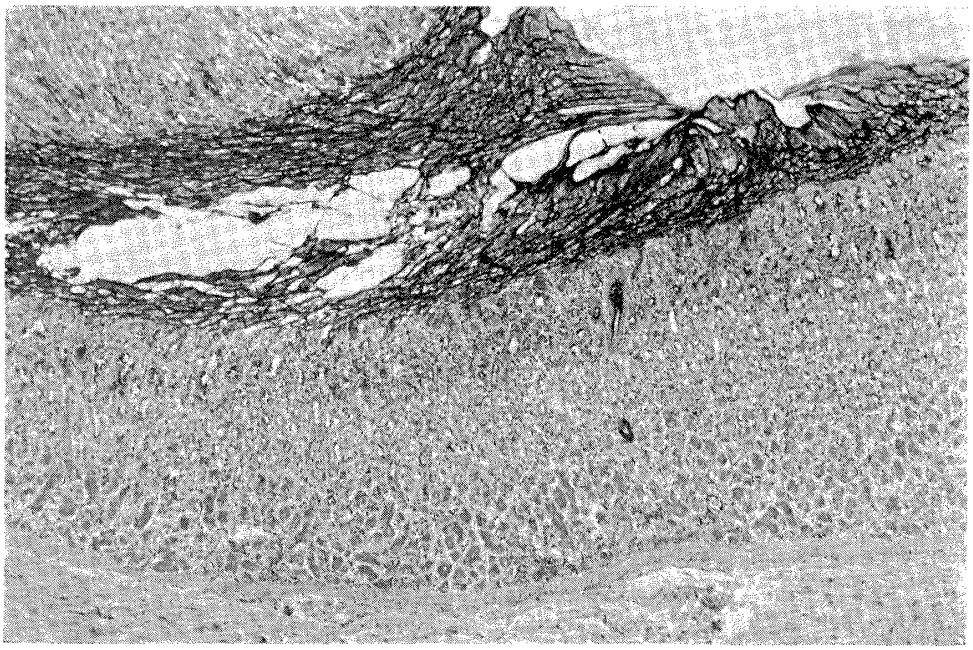

Photo. 24 Gastric mucosa in No. 2., 6-year-old child dying in a few hours after onset, showing hypersecretion of mucus covering the mucosa. Edema and inflammatory cell infiltration does not present in the mucosa and submucosa.
Alcian blue-PAS-Masson's trichrome. ×48.

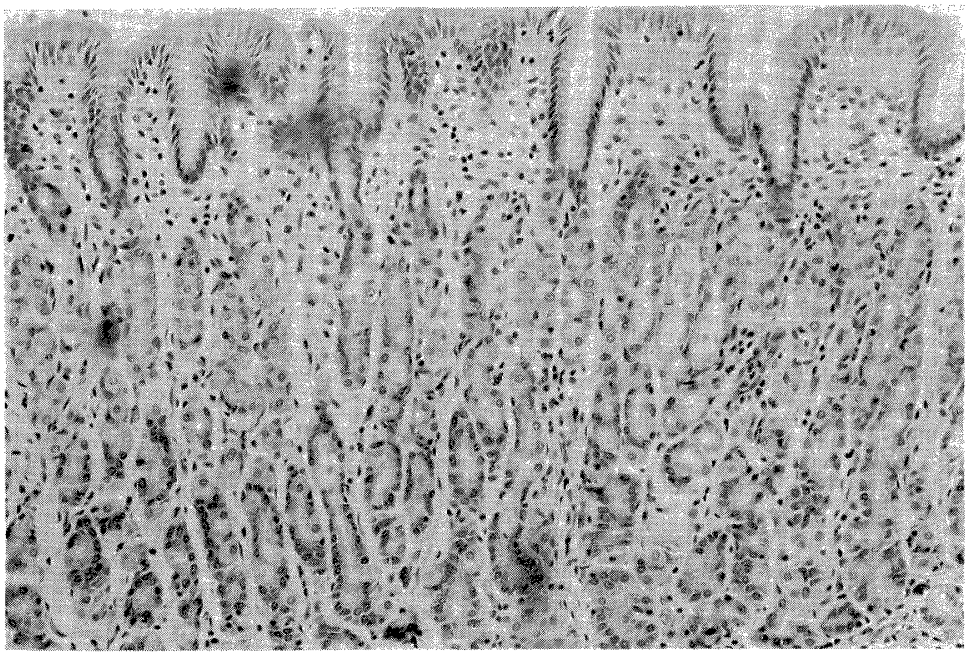

Photo. 25 Gastric mucosa in No. 13., 2-year-old child of 24 hours.
Note absence of edema or hypercellularity of the lamina propria. Some parietal cells show intracytoplasmic vacuole. In this case, the surface epithelium is almost intact without desquamation.
H. & E. ×180.

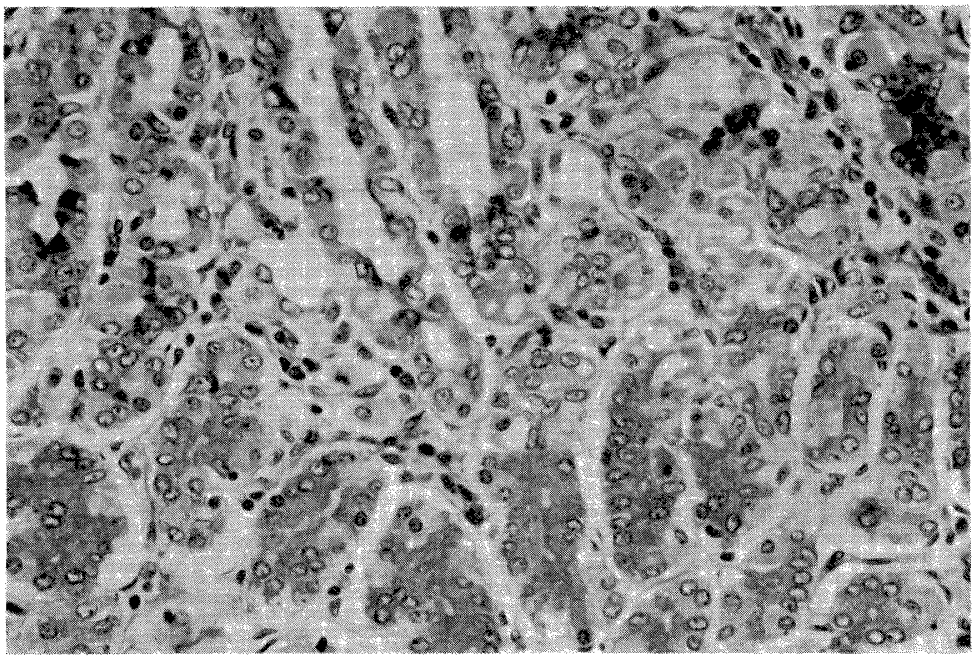

Photo. 26 Neck portion of gastric gland in No. 2., the same case as Photo. 24. Parietal cells disclose large vacuoles in the cytoplasm.
H. & E. ×360.

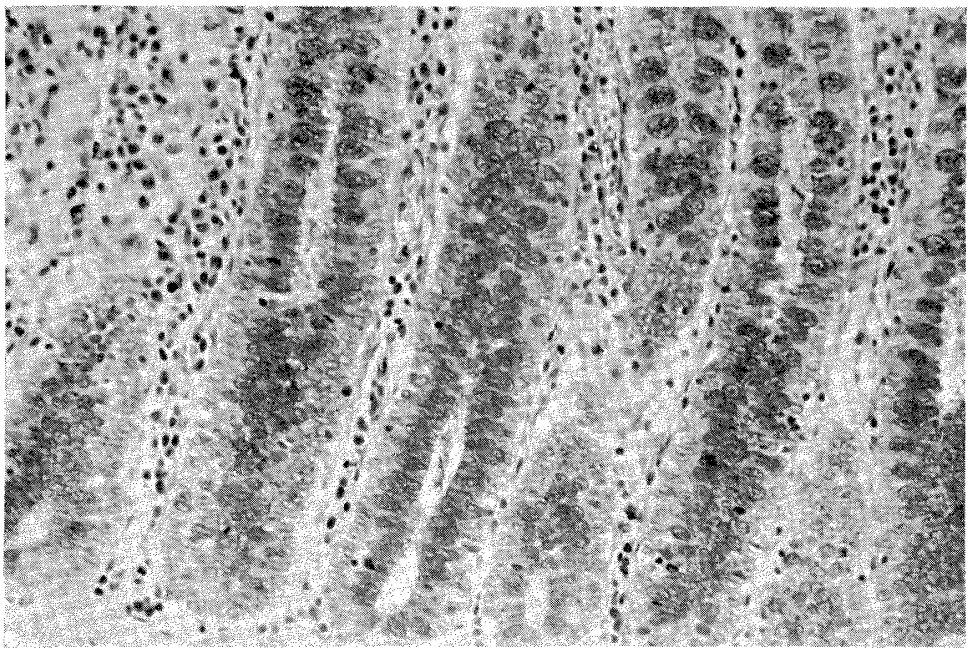

Photo. 27 Colonic mucosa in No. 2., 6-year-old child, dying in a few hours after onset. Goblet cells are filled with a large amount of mucus. Secretion of mucin already begins.
Alcian blue-PAS-Masson's trichrome. ×240.

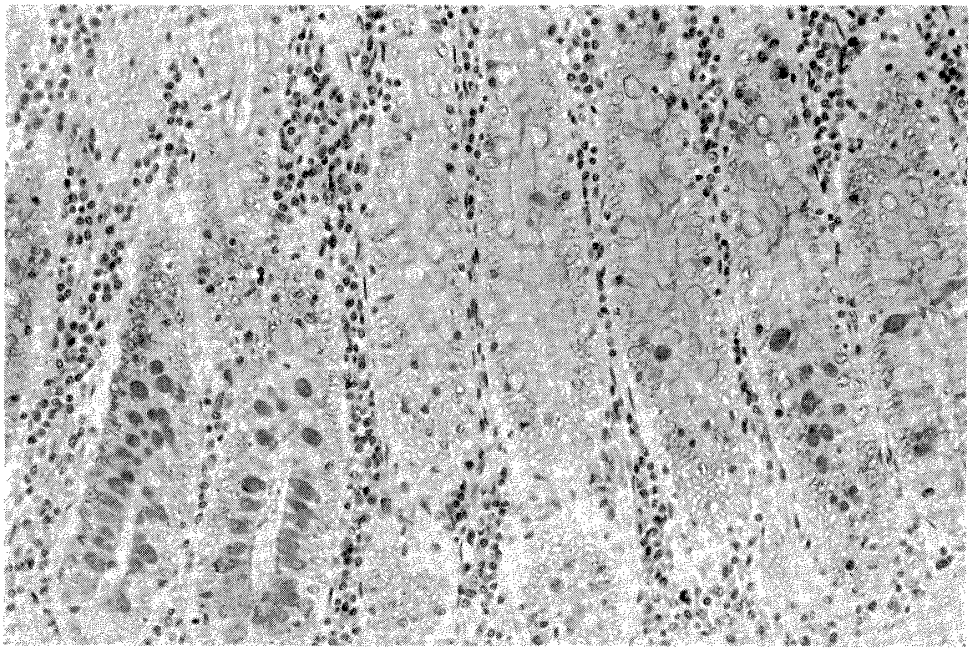

Photo. 28 Colonic mucosa in No. 9., 4-year-old child of 12 hours. Hypersecretion of goblet cells. In the upper two third of the crypts, the goblet cells are pale and reticular because of early secretory activity. Those of the lower third preserve the mucus. The lamina propria shows a moderate infiltration of inflammatory cells.
Alcian blue-PAS-Masson's trichrome. ×240.

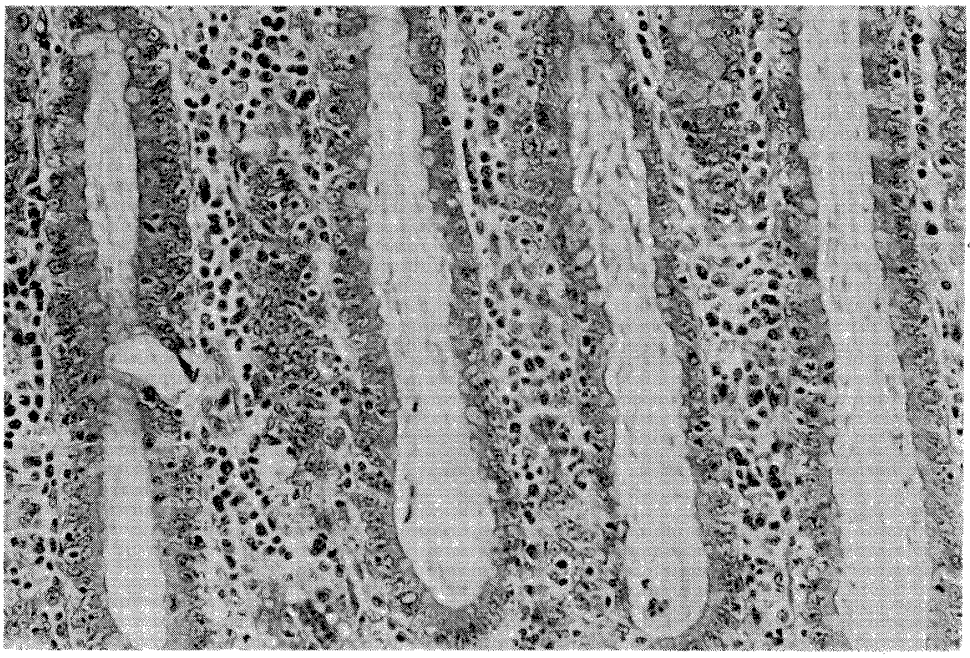

Photo. 29 Colonic mucosa in No. 10., 3-year-old child of 13 hours. The lumina of the glands are dilated and filled with mucus. An amount of mucin in the cytoplasm is decreased by completion of secretion. The lamina propria shows advanced hypercellularity. Some lymphocytes migrate into the glandular epithelium.
Alcian blue-PAS-Masson's trichrome. ×240.

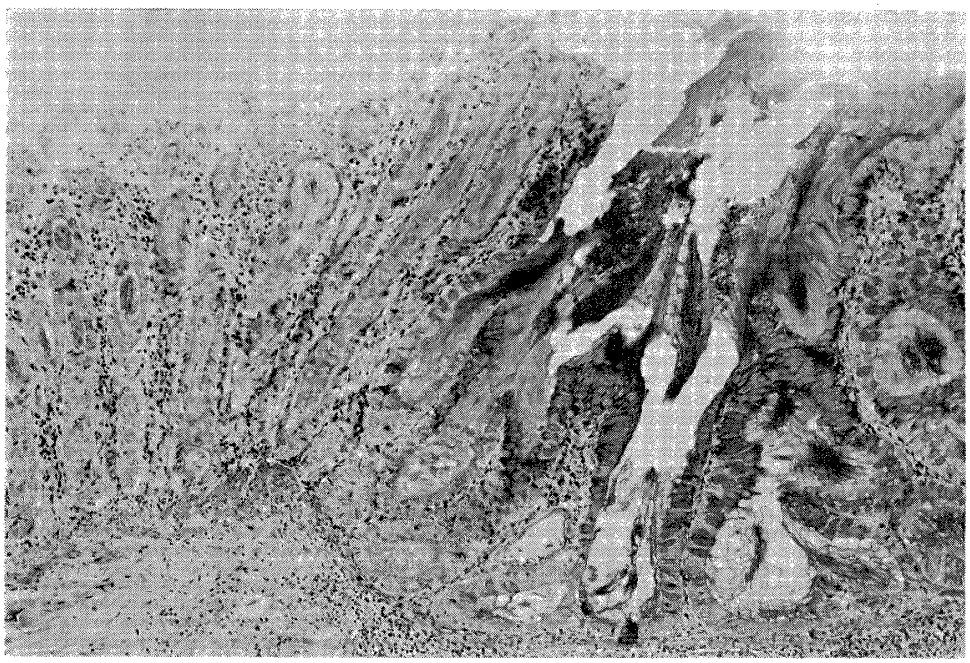

Photo. 30 Colonic mucosa in No. 20., 2-year-old child of 7 days.
Note irregular dilatation of the crypts associated with hypersecretion of mucus. Cell infiltration is prominent in the mucosa.
Alcian blue-PAS-Masson's trichrome. ×90.

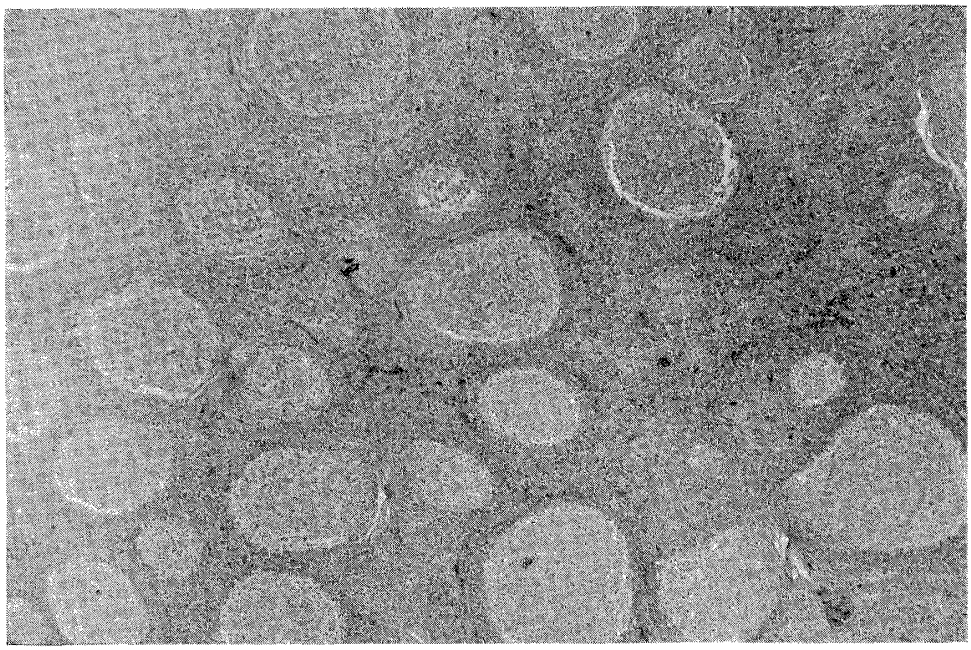

Photo. 31 Mesenteric lymphnode in No. 7., 8-year-old child of 10 hours. Note increase of number and size of follicles with marked hyperplasia of germinal center.
H. & E. ×36.

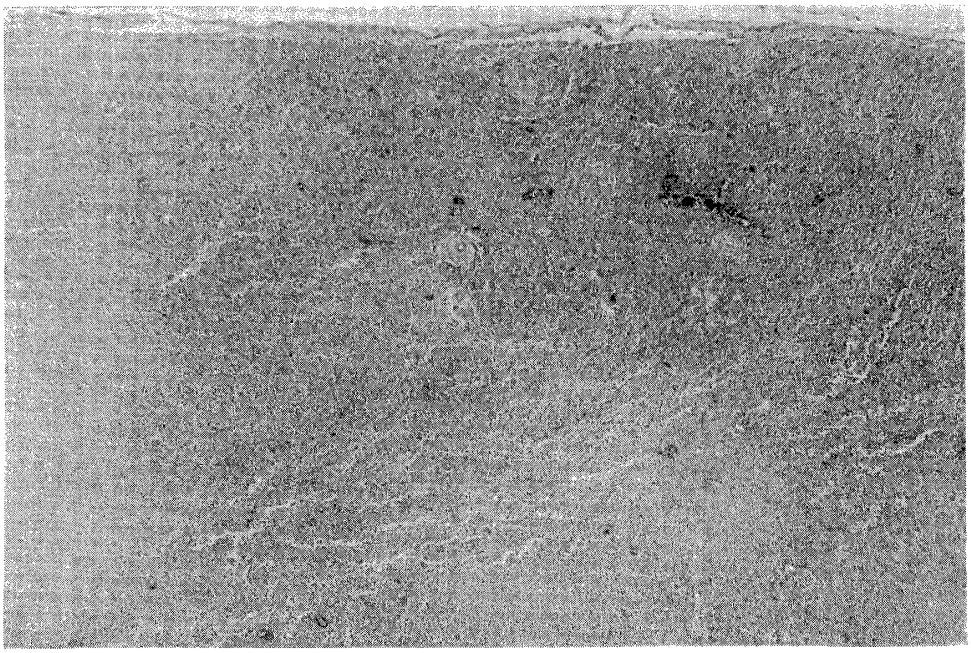

Photo. 32 Mesenteric lymphnode in No. 14., 5-year-old child of 30 hours. Note diffuse proliferation of lymphocytes. There is no hyperplasia of lymph follicles.
H. & E. ×36.

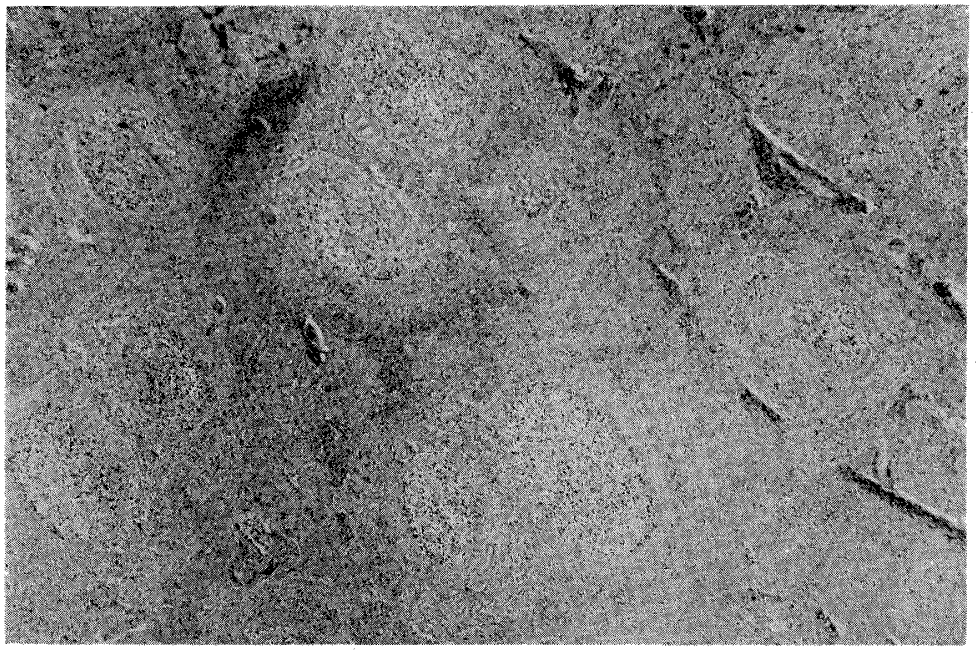

Photo. 33 Spleen in No. 15., 3-year-old child of 32 hours.
Note hyperplasia of follicles especially of germinal centers which are necrotic. Margins of follicles are indistinct.
H. & E. ×36.

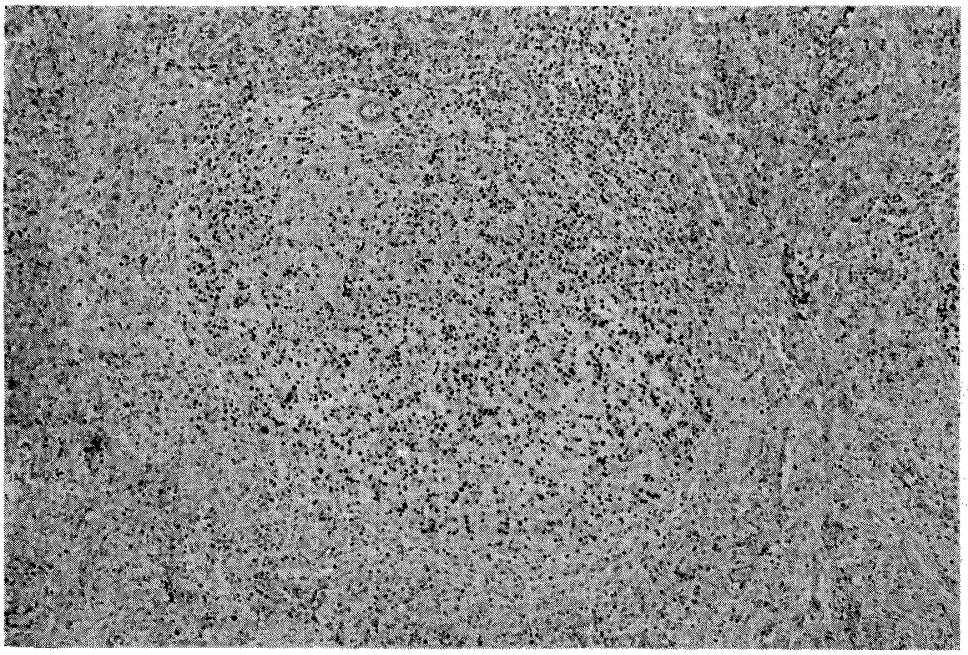

Photo. 34 Magnituded view of Photo. 33. Germinal centers show hyperplasia with destruction of tissue. Note resolution of red pulps.
H. & E. ×120.

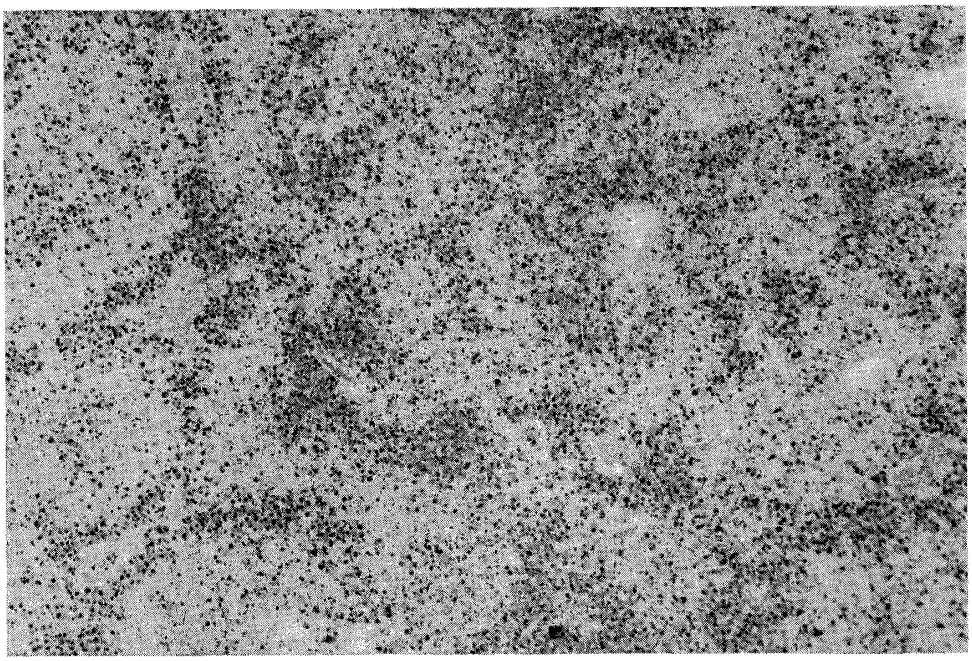

Photo. 35 Liver with fat stain in No. 15. Distribution of fatty metamorphosis is peri- and centroacinar. Degree of metamorphosis is moderate.
Oil red O stain. ×36.

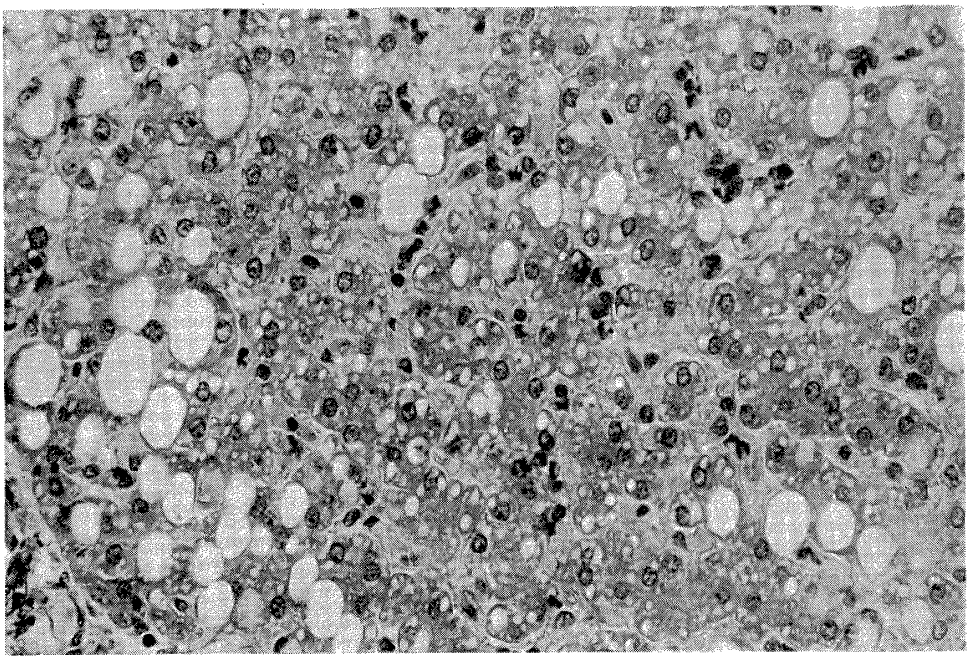

Photo. 36 Liver in No. 13., 2-year-old child of 24 hours. Note advanced fatty metamorphosis. Large and small fat droplets are seen in the hepatic cells. Congestion is mild in this specimen.
Heidenhain's azocarmine. ×360.

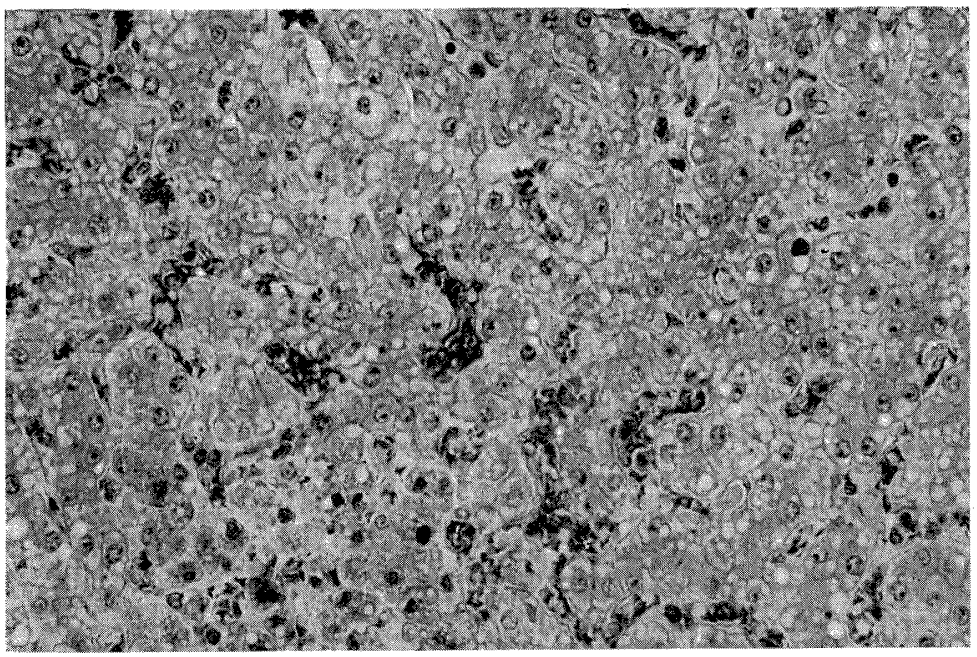

Photo. 37 Liver in No. 15., 3-year-old child of 32 hours. Fat droplets of metamorphosis are medium in size. Congestion is seen in this case.
Heidenhain's azocarmine. ×360.

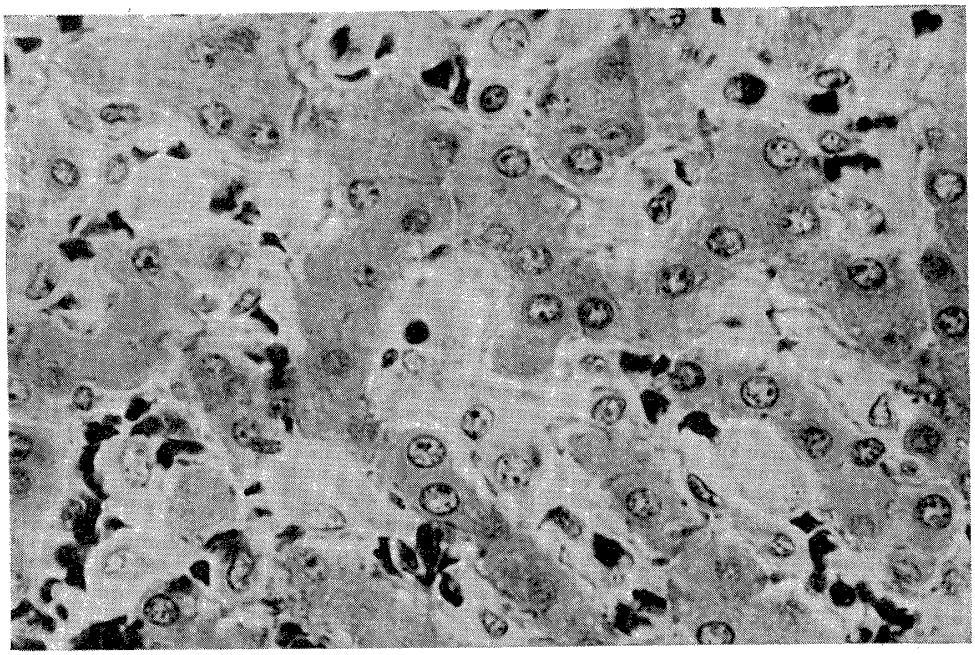

Photo. 38 Liver in No. 7., 8-year-old child of 10 hours.
Fatty metamorphosis is mild. Mobilization and hyperplasia of the Kuppfer's cell are noted.
Heidenhain's azocarmine. ×720.

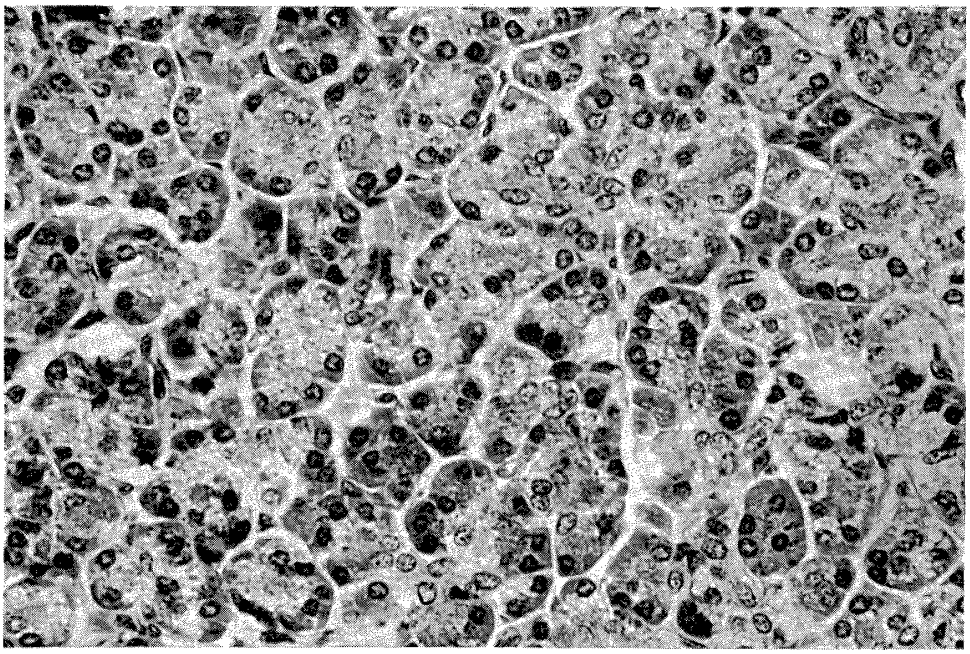

Photo. 39 Pancreas in No. 4., 63-year-old male of 7 hours.
The apical portion of the acinar cells is foamy or finely vacuolated. It looks like the state just after secretion of zymogen granules.
Heidenhain's azocarmine. ×480.

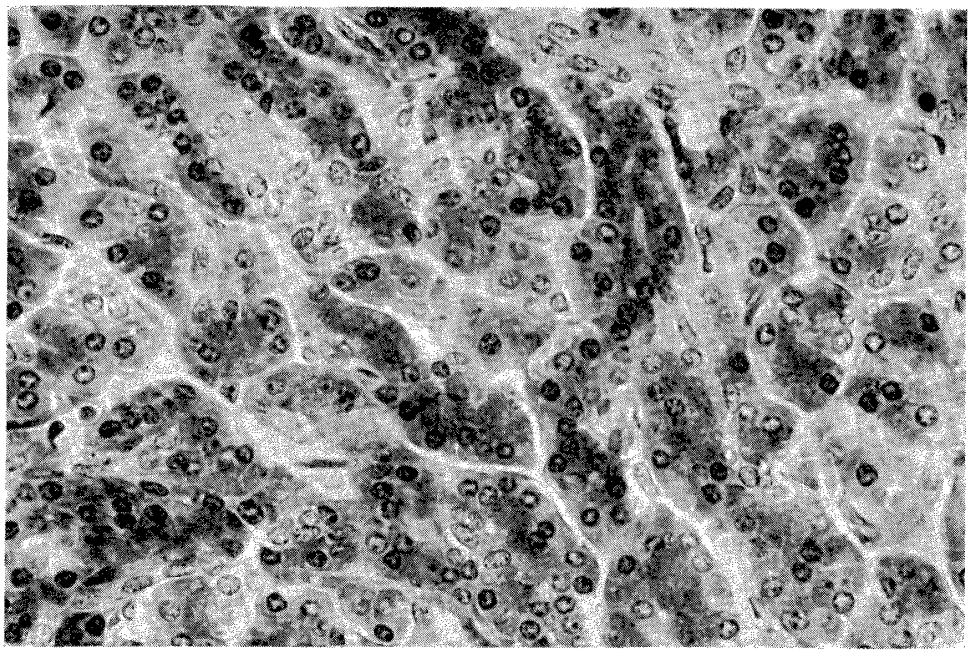

Photo. 40 Pancreas in No. 6., 20-year-old female of 10 hours.
A large amount of zymogen granules is accumulated in the cytoplasm of the acinar cells.
Heidenhain's azocarmine. ×480.

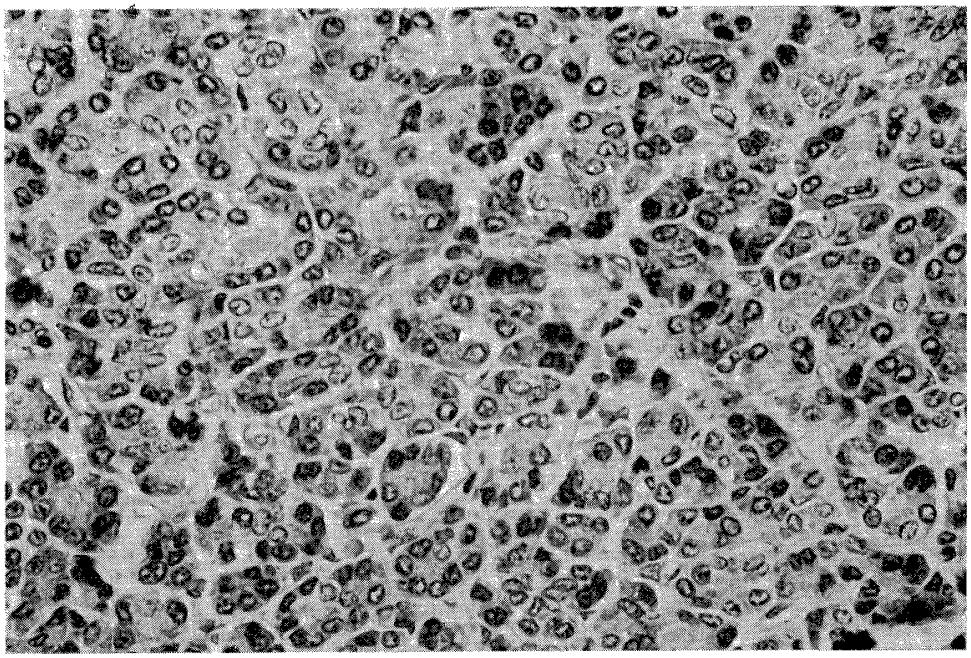

Photo. 41 Pancreas in No. 20., 2-year-old child of 7 days.
The acinar cells are atrophic, and do not contain zymogen granules. Somewhat dilated lumina of the acini are filled with blue stained material.
Heidenhain's azocarmine. ×480.

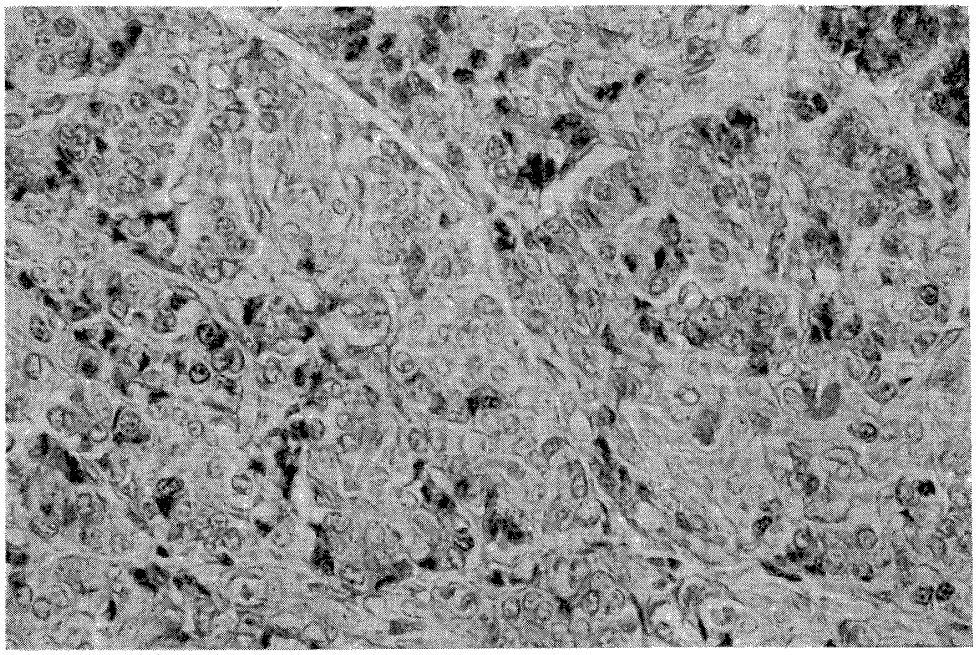

Photo. 42 Pancreas in No. 21., 6-month-old infant of 3 weeks.
The acinar cells show atrophy, surrounded by inter-acinar fibrosis. The lumina of the acini contain dense fluid.
Heidenhain's azocarmine. ×480.

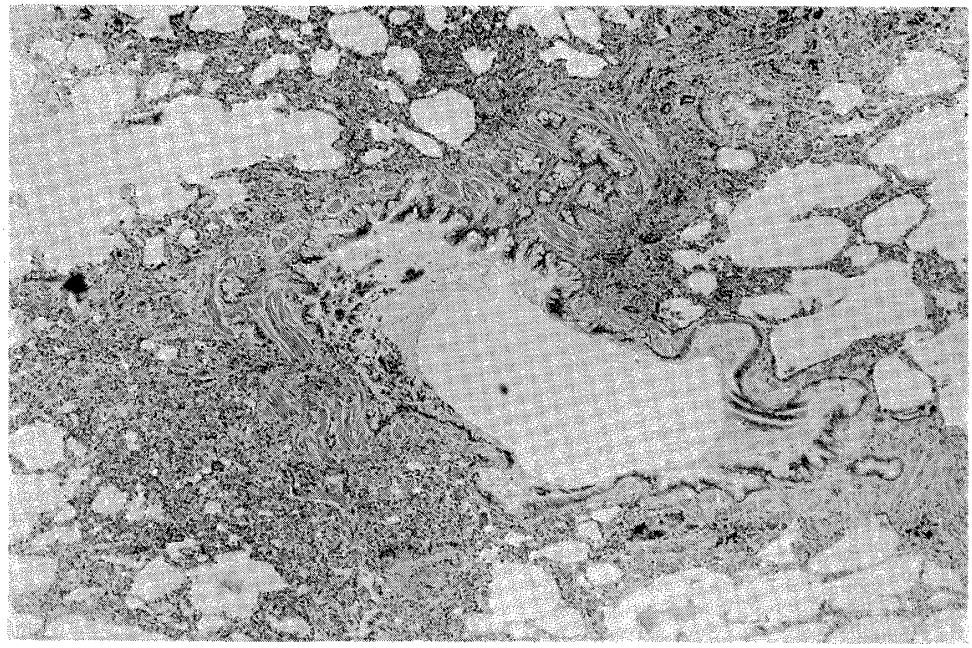

Photo. 43 Lung in No. 18., 14-year-old girl of 40 hours.
The Bronchial mucosa shows hypersecretion of mucus from goblet cells. The lumen of the bronchus contains fluid admixed with mucus. Inflammatory cell infiltration due to inhalation of gastric content is seen in the peribronchial region.
H. & E. ×48.

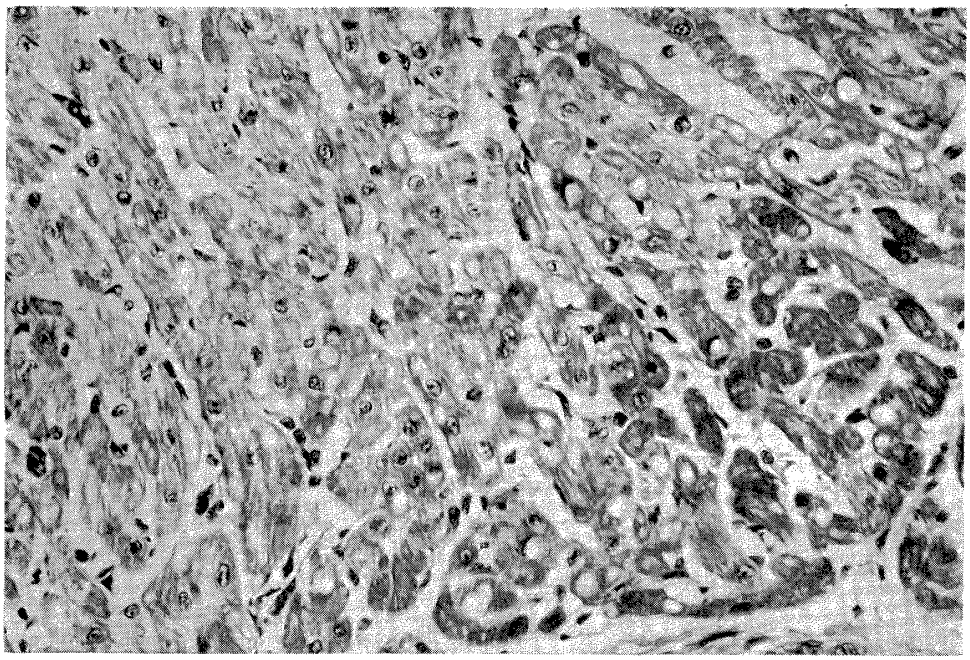

Photo. 44 Heart in No. 19., 1-year-old child of 41 hours.
Note large vacuolation of myocardial cells.
Heidenhain's azocarmine, ×360.

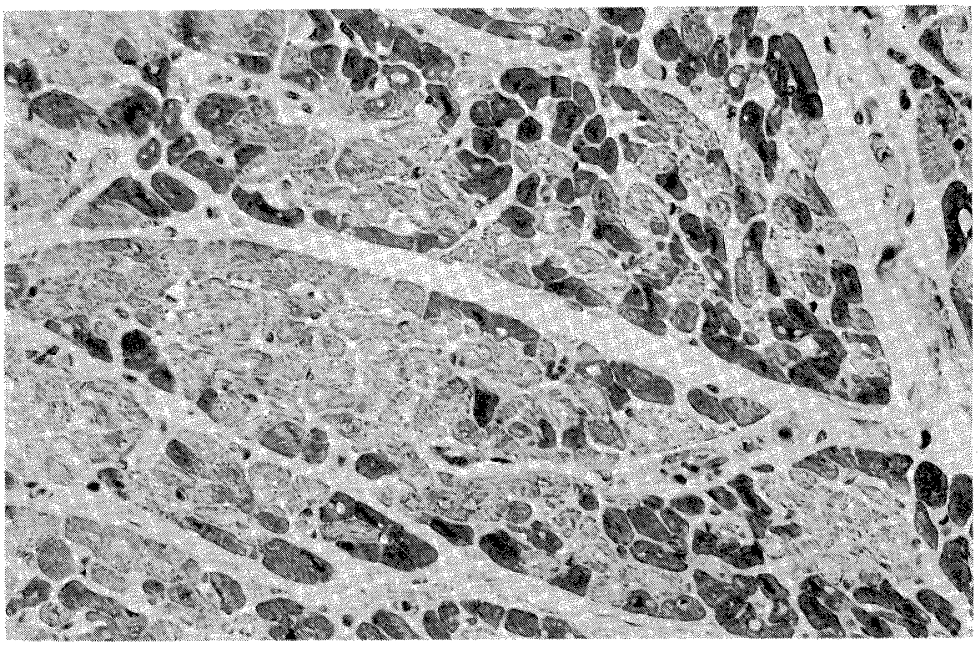

Photo. 45 Heart in No. 18., 14-year-old girl of 40 hours.
Note irregularity of myocardial cells in staining by Heidenhain's azocarmine stain. There is no evidence of necrosis of myocardium or myocarditis.
Heidenhain's azocarmine. ×360.

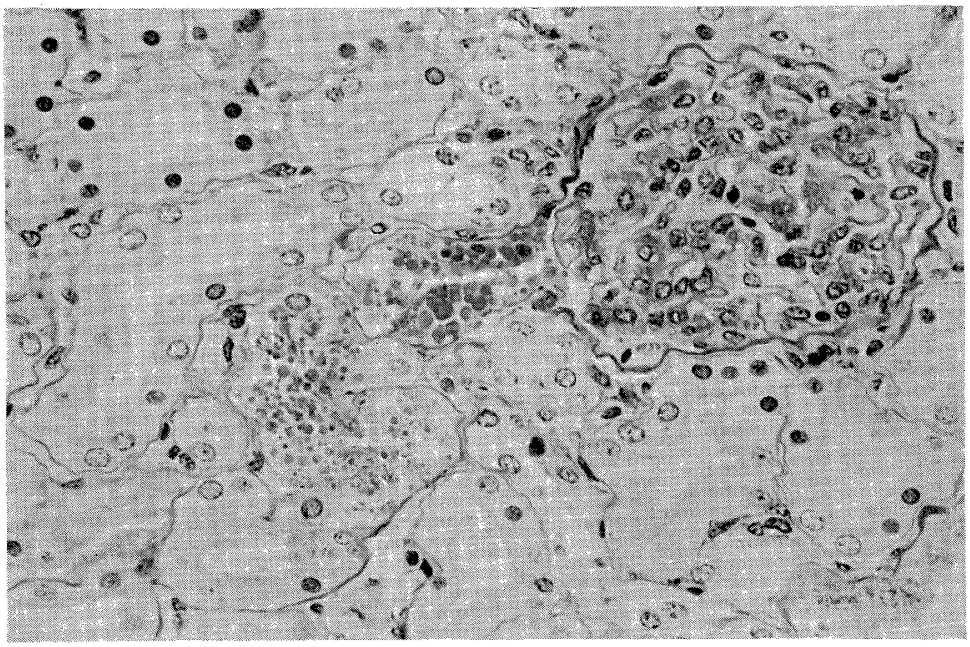

Photo. 46 Kidney in No. 7., 8-year-old child of 10 hours.
The neck portion and proximal regions of the tubules disclose hyaline droplet formation. Droplets are conglomerated in the neck portion.
PAS. ×480.

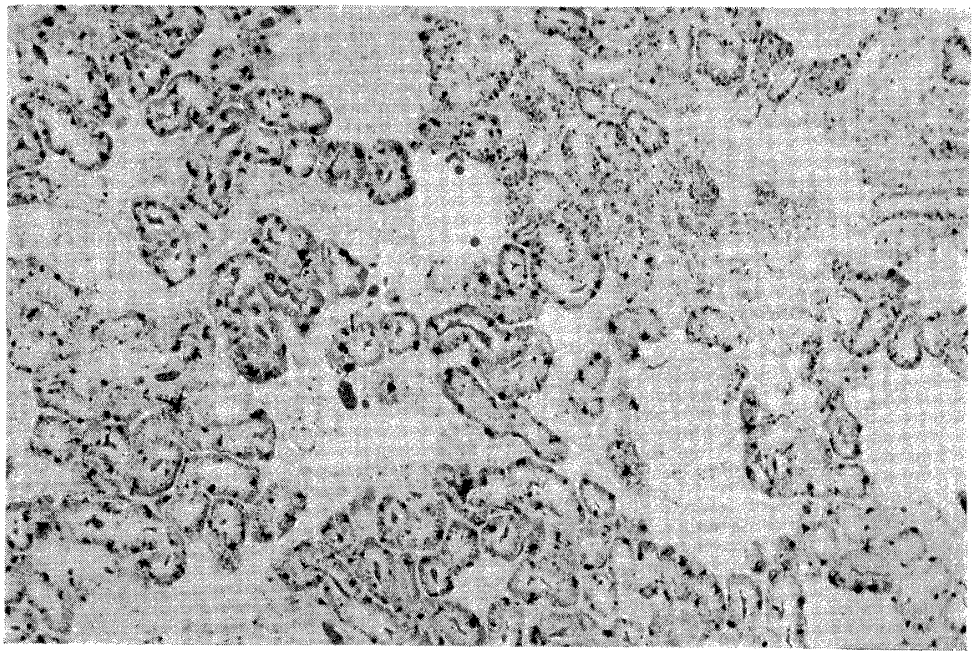

Photo. 47 Kidney in No. 17., 8-year-old child of 40 hours.
Fatty change of the tubular epithelium is severe, and predominant in the proximal tubules.
Oil red O stain. ×90.

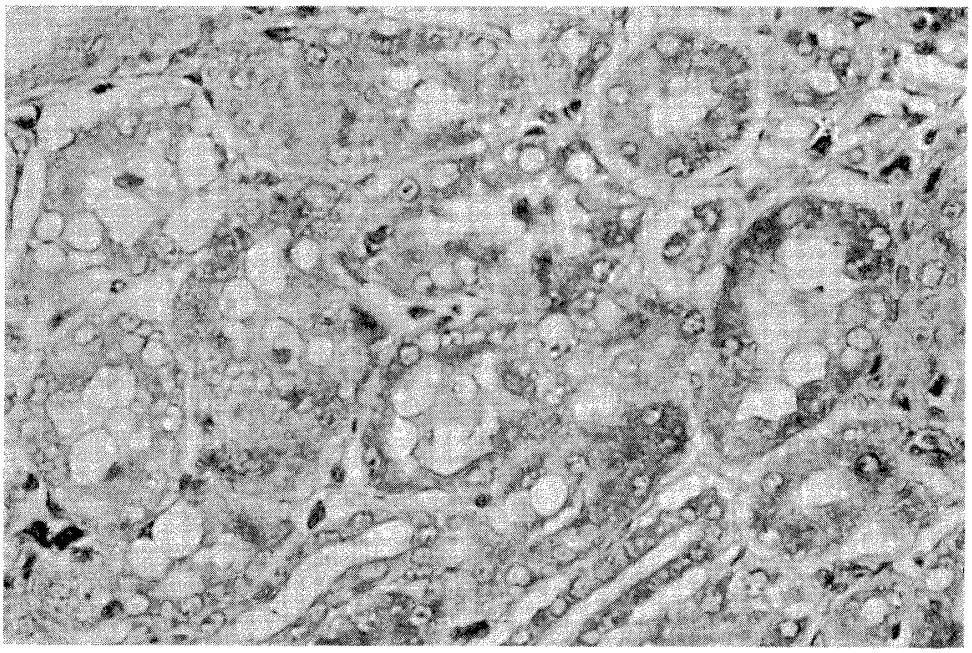

Photo. 48 Kidney in No. 21., 6-month-old infant of 3 weeks.
Hypokalemic vacuolar change is advanced in the cases of 7 days and 3 weeks.
Heidenhain's azocarmine. ×360.

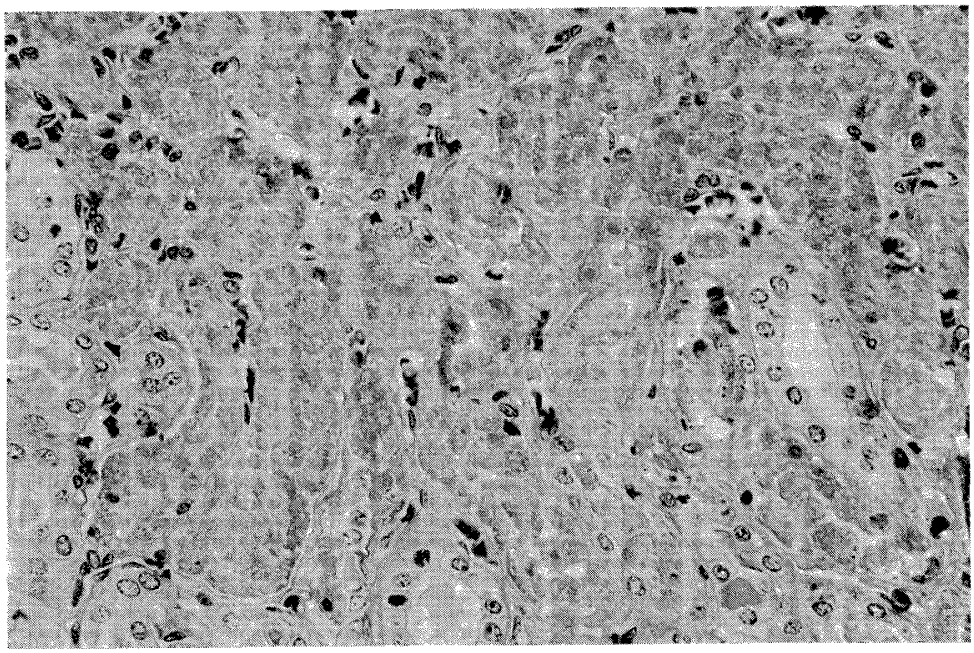

Photo. 49 Kidney in No. 17., 8-year-old child of 40 hours.
Note patchy necrosis of the cortex. The proximal tubules are more affected.
Heidenhain's azocarmine. ×360.

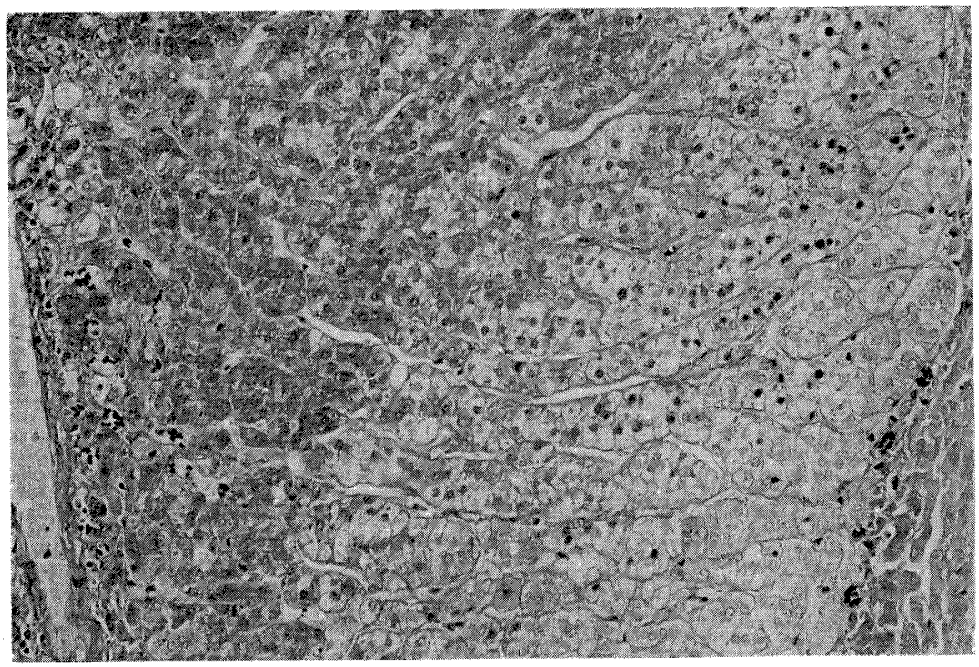

Photo. 50 Adrenal gland in No. 4., 63-year-old male of 8 hours.
The outer half of the cortex shows clear and foamy change of the cortical cells and the inner half shows compact like cells. Note no evidence of severe damage of the parenchymal cell or inflammatory cell infiltration in the interstitium.
Heidenhain's azocarmine. ×360.

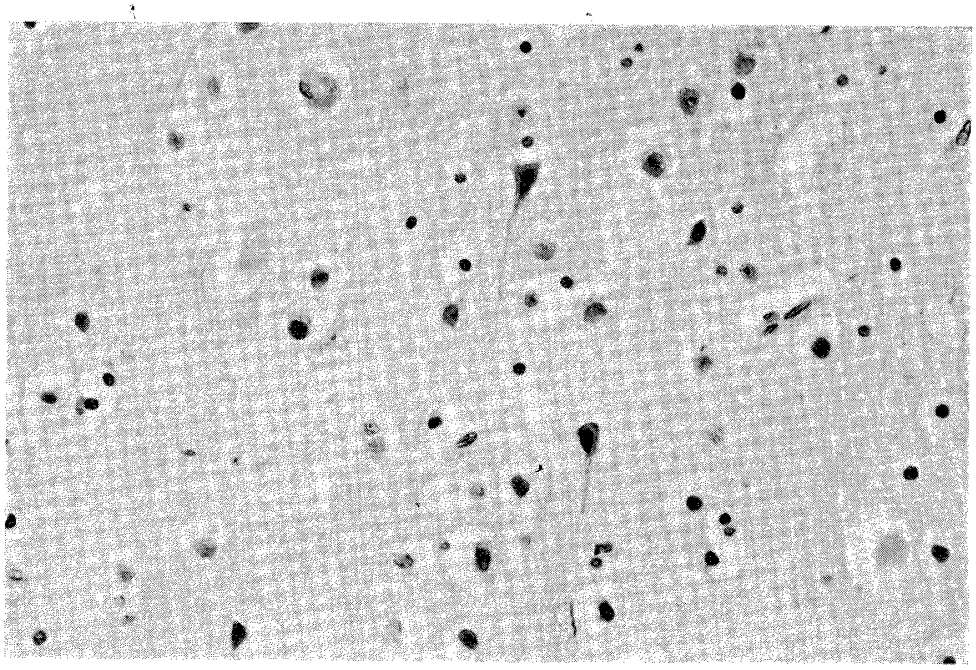

Photo. 51 Brain in No. 13., 2-year-old child of 24 hours.
Note retraction of the nerve cell body and perinuclear edema.
H. & E. ×360.

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

Vol. 5 No. 3, 4 December, 1977

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Published by

JAPANESE SOCIETY OF TROPICAL MEDICINE

c/o Institute for Tropical Medicine, Nagasaki University

12-4 Sakamoto-machi, Nagasaki, 852, Japan