protein', and in the presence of the 'acute burn serum inhibitor' as shown in Figure 2B. Figure 2C suggest that, when pretreated with the 'acute burn serum inhibitor', then washed and allowed to migrate in fresh MEM, normal lymphocytes failed to migrate. The results of these experiments, summarized in Table IB, suggest that the 'acute burn serum inhibitor' reacts with the lymphocytes. The magnitude of the migration inhibition varied from one patient to another.

Discussion. When incorporated into the growth medium, the 'acute burn serum inhibitor' depressed the in vitro growth of rabbit heart fibroblasts. If added to the Earle minimal essential medium, it inhibited the migration and growth of the normal lymphocytes. Lymphocytes of acute thermally injured patients migrated at slower rate than the normal lymphocytes. The speed of migration of these lymphocytes varied from one injured patient to another, and possibly depended on the magnitude of the thermal injury. These observations indicate the release of cytotoxic antigen into the blood of the injured patients, and suggest an impairement of cellular immunity in the acute thermally injured patients, which is in agreement with the findings from other laboratories[11,12]. MESSERSCHMIDT[11] showed disorders of antibody formation in mice following burns and whole body irradiation, and QUISOMORIO[12] described the occurence of rheumatoid factors, antinuclear antibodies and antiepithelial antibodies in thermal injury.

It is obvious that a toxic antigen released into the circulation as a result of thermal or other type of insult, i.e. trauma, viral or neoplastic, will bind at the surface or on the plasma membrane of the lymphocytes leading to cytotoxic reactions in immunologically specific and non-specific cells. The evidence cited above suggests that the 'acute burn serum inhibitor' acted directly on the lymphocytes. This interaction could lead to the blocking of receptors on the lymphocytes which are necessary for recognition of foreign antigens, and the induction of a non-responsive, depressed state in the reticuloendothelial system of the victims[13,14].

A) Migration of human lymphocytes from acute thermally injured patients

Lymphocytes from	Control migration (%)
Normal	100
Thermally injured patients	
D.A.	62
B.H.	39
W.W.	70
T.R.	10
K.L.	40

B) Migration inhibition of normal human lymphocytes

Factor in medium	Inhibition (%)
Normal saline	0 (10)[a]
Normal serum	0 (15)
Toxic glycoprotein (50 µg)[b]	98 (14)
Toxic glycoprotein (25 µg)	85 (9)
Toxic glycoprotein (5 µg)	75 (10)
Acute burn serum inhibitor (5 µg)	
D.A.	77 (5)
W.W.	95 (8)
B.H.	85 (10)
Acute burn serum (0,05 ml)	
D.A.	85 (6)
W.W.	85 (6)
T.R.	76 (10)
K.L.	60 (8)

[a] Number in paranthesis are munber of aliquots assayed. [b] Amount used per ml of medium. All the sera were used after inactivation at 56 °C for 30 min.

Résumé. Un facteur cytotoxique fut obtenu du serum des malades qui ont été fortement brûlés. Il a produit l'inhibition et l'agglutination des fibroblastes cardiaques du lapin et a inhibé la migration des lymphocytes humains.

A. A. HAKIM

The Biochemistry Research Laboratories of the I.T.R., 259 DMP School of Medicine. University of Illinois Medical Center, Chicago (Illinois 60680, USA), 12 January 1973.

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The Fate and Effects of *Salmonella* Flagellin in Neonatal Rat Intestine

The biological distribution and fate of antigens injected s.c. or i.v. has been extensively studied[1-3]. In contrast, there is very little information about the fate or effect of antigens which occur naturally, particularly in the neonatal period. Studies of antigens entering through the surfaces of the intestine and lungs have been limited to immunologically mature animals[4-7] and studies of antigen in neonatal animals have been limited to injected material[8,9]. Antigenic proteins as large as ferritin are absorbed through the intestinal mucosa of neonatal animals[10,11], and this absorption has selectivity and is age-related[12]. We have started to study the immunological effect and the biological fate of intestinally absorbed macromolecules in neonates.

Materials and methods. Spraque-Dawley rats aged 1–20 days were used. The mothers received in drinking water potassium iodine, 1 mg/l, for 7 days prior to parturition and until the neonates were killed. Monomeric flagellin was prepared from *Salmonella* Derby, American Type Culture Collection, Maryland, and labelled with carrier free I^{125} by the method of ADA et al.[13]. 25 µg labelled flagellin in 0.1 ml of a diluted suspension of colloidal carbon (marker substance) were delivered into the distal half of the stomach through a polyethylene tube. Rats studied were 1, 5, 10, 15 and 20 days of age. Rats were killed at times varying from 3 to 120 h after antigen administration. There were 6 to 10 rats killed at each time interval. The intestine with attached mesentery and lymph nodes, spleen, thymus, liver and lungs were rinsed in saline three times to remove free iodide, counted using a scintillation crystal and Baird-Atomic spectrometer, fixed in 10% formalin and then prepared as microscopic sections for autoradiography with Kodak NTB 3 emulsion. Comparisons were made with the distribution

Table I. Mean and standard errors of percent of dose (cpm organ/cpm total dose $\times 100$) of I^{125} in spleen, liver and intestine at various times after intragastric administration of I^{125} labelled *Salmonella* Derby flagellin to rats aged 1 to 10 days of age

Organ	Age of animals (days)	Time after administration of labelled flagellin (h) 3	9	10	24	72	120
Spleen	1	1.0 ± 0.12	1.4 ± 0.21	1.1 ± 0.10	1.1 ± 0.10	0.06 ± 0.02	0.01 ± 0.02
	5	1.6 ± 0.20	1.9 ± 0.10	2.1 ± 0.30	1.2 ± 0.10	ND	ND
	10	1.7 ± 0.10	1.1 ± 0.10	0.7 ± 0.12	0.4 ± 0.10	0.05 ± 0.01	ND
	15	0.7 ± 0.08	0.3 ± 0.04	0.3 ± 0.06	0.2 ± 0.01	0.05 ± 0.01	0.01 ± 0.01
	20	1.2 ± 0.10	0.7 ± 0.05	1.2 ± 0.10	0.6 ± 0.01	ND	ND
Liver	1	8.0 ± 1.4	5.5 ± 0.90	3.0 ± 0.60	2.7 ± 1.3	0.1 ± 0.06	0.05 ± 0.01
	5	6.4 ± 0.80	9.0 ± 0.60	11.0 ± 2.0	4.0 ± 0.30	ND	ND
	10	10.0 ± 4.0	4.8 ± 1.7	ND[a]	1.8 ± 0.70	0.15 ± 0.15	ND
	15	3.8 ± 1.5	0.5 ± 0.08	1.7 ± 0.30	0.8 ± 0.08	0.05 ± 0.01	0.02 ± 0.01
	20	6.7 ± 2.6	5.5 ± 0.10	0.9 ± 0.02	0.9 ± 0.19	ND	ND
Intestine	1	26.9 ± 7.7	20.1 ± 2.0	18.6 ± 4.3	4.5 ± 1.4	0.6 ± 0.02	0.2 ± 0.02
	5	37.2 ± 9.0	41.8 ± 4.1	41.6 ± 5.1	28.1 ± 3.4	ND	ND
	10	31.3 ± 7.2	32.1 ± 7.4	29.6 ± 6.0	17.5 ± 1.3	8.2 ± 1.0	ND
	15	15.4 ± 3.1	12.3 ± 0.90	15.6 ± 2.7	9.6 ± 0.20	4.4 ± 1.5	1.5 ± 0.17
	20	32.1 ± 9.2	37.4 ± 1.7	30.0 ± 5.3	24.1 ± 7.0	ND	ND

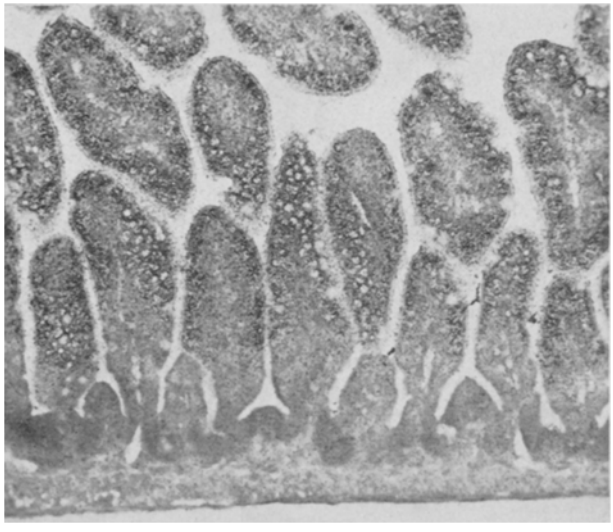
There were 6–10 animals in each group. ND, Not done.

of the same doses of labelled flagellin injected intraperitoneally and with the distribution of orally administered and similarly labelled bovine serum albumin (BSA). Rats given 25 µg unlabelled flagellin into the stomach were bled 20 days later. These animals were reimmunized with 100 µg flagellin i.p. 60 days after the intraintestinal antigen, and bled again after 20 days from the i.p.-immunization. The sera were tested for anti-flagellin titers using flagellin coated sheep erythrocytes perpared by the $CrCl_3$ method[14].

Results and discussion. The data derived from the γ-counting of individual organs were expressed as per cent of injected dose (cpm of organ/cpm of injected dose $\times 100$) and are shown for spleen, liver and intestine with attached mesentery in Table I. In the 1- and 5-day-old rats, the persistent radioactivity increased in the spleen for 6 to 10 h. A similar slow increase in radioactivity occurred in the liver and less certainly in the intestine and mesentery of 5-day-old rats. In all other age groups and organs, the proportion of retained radioactivity decreased with time after the first measurement at 3 h.

The greatest proportion of persistent radioactivity was in the intestine and attached mesentery, then in order, in the liver, lungs and spleen. Similar patterns were seen after i.p. injection of labelled flagellin, except that greater amounts were retained by liver and spleen, Table II. After intragastric administration of labelled BSA, the distribution was similar to that of flagellin, but there was no increase in the radioactivity between 3 and 6 h.

The autoradiographs showed both focal and diffuse localization of radioactivity. Initially there were dense accumulations of silver grains over mucosal cells with unique, giant vacuoles in the ileum from day 1 to day 15



Photomicrograph of an autoradiograph of a section of ileum of a 1-day-old rat killed 6 h after delivery of I^{125}-labelled *Salmonella* Derby flagellin into the stomach. Radioactivity is associated with mucosal cells on the villi which are notable for very large 'supranuclear' vacuoles. Methylgreenpyronin stain. ×200.

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after birth (Figure). By day 20, very few of the mucosal cells had these vacuoles, and they no longer retained label. 10 or more h after administration, dense accumulations of label were found in the submucosa within villi and adjacent to the muscularis. By 5 to 15 days Peyer's patches were maturing and similar 'hot spots' could be seen in areas relatively close to the overlying mucosa, but not in the developing follicular areas. In mesenteric nodes and spleen, only diffuse labelling was found over the lymphoid areas. In contrast, the spleens of rats receiving i.p. injections contained scattered foci of intense radioactivity in developing transitional zones and superficial portions of nascent periarteriolar lymphocyte cuffs. Even after i.p. injections, there was dense label over the vacuoles of the mucosal cells.

The results of the agglutinin assays for anti-flagellin antibody are shown in Table III. Only rats given intraintestinal flagellin at 5 days of age developed measurable antibodies. One day old rats challenged i.p. 2 months after antigen administration developed no antibody within two weeks of i.p. challenge, while rats given oral flagellin at later times did have a response.

This work indicates that intraintestinal flagellin, in the doses used in these experiments, can have an effect on the systemic immune system during at least the first 5 days of a rat's life, possibly tolerance on day 1 and certainly immunization on day 5. At these times there was a slow and prolonged uptake of radioactivity from labelled flagellin by the spleen, and at 5 days also by the liver and intestine with attached mesentery, suggesting macromolecular absorption and transportation. At later times, when intraintestinal antigen had no measurable immunological effect, the radioactivity from labelled antigen decreased in all organs from the first time studied, suggesting metabolism of digested material.

Nothing in this work proves that the radioactivity was necessarily associated with antigen, although this has been proved with this system in rats injected s.c. or i.p.[1-3, 8, 9, 15]. It is known that the major problem associated with use of iodinated antigens in neonatal rats is excessive loss in processing of macromolecular material, rather than misleading retention of metabolic breakdown products of free iodide[16].

The most striking observation from the study of the autoradiographs was the association of label with those ileal mucosal cells which had greatly enlarged vacuoles. The presence of these cells was clearly age-related as they had virtually disappeared by day 20. 'Supranuclear' vacuoles and their role in macromolecular absorption in neonatal rats has been described before[10, 11], as has the appearance within these vacuoles of protein injected into the peritoneum[17]. We cannot determine with light microscope autoradiography whether the 'hot spots' found in the lamina propria were due to reticulo-endothelial cells or to antigen-reactive lymphocytes. The absence of such areas of dense radioactivity in the lymph nodes and spleen of neonatal rats receiving antigen via the intestine may be due only to the smaller amount of antigen reaching those organs than in rats receiving labelled antigen intraperitoneally.

This work proves that macromolecular antigens can be absorbed and cause a systemic reactivity in rats. It has been proposed that under usual circumstances secretory IgA antibodies block absorption of intestinal antigens and thus prevent systemic antibody response[18]. If our results are due to an absence of anti-flagellin antibodies by 5 days of age, it becomes necessary to explain spontaneous appearance of such antibodies before 10 days of age. An alternative explanation might lie in the function of the uniquely vacuolated cells in the ileal mucosa of the neonatal rats, cells which appear to gain selectivity in absorption from different proteins at different ages[12].

Table II. Mean and standard error of percent of dose of I^{125} in spleen, liver, and intestine at intervals after i.p. injection of labelled flagellin to rats aged 1 and 8 days

Organ	Age of animals (days)	Time after immunization (h) 6	10	24
Spleen	1	ND	3.7 ± 0.45	1.7 ± 0.17
	8	3.2 ± 2.6	1.7 ± 0.20	0.4 ± 0.02
Liver	1	ND	10.5 ± 0.10	8.7 ± 1.6
	8	8.0 ± 2.0	11.4 ± 1.0	2.6 ± 0.80
Intestine	1	ND	27.2 ± 4.2	20,5 ± 2.0
	8	12.8 ± 2.4	12.2 ± 2.5	8.9 ± 0.80

ND, Not done.

Table III. The mean titers of antiflagellin antibodies as determined by hemagglutination of $CrCl_3$ treated and flagellin coated sheep red cells

Age given antigen per intestine (days)	Antiflagellin titer after 20 days (range)	Antiflagellin titer 20 days after i.p. challenge (range)
0	0	0
5	1:40 (4–128)	1:170 (16–256)
10	0	1:50 (32–64)
15	0	1:1400 (1024–2048)
20	0	1:100 (64–128)
Adult	–	1:800 (512–1024)

Serum was obtained 20 days after administration of 25 µg flagellin into the stomach. The rats were rested 2 months, then given 100 µg flagellin i.p., and bled again after 20 days. There were 10 animals in each group.

Zusammenfassung. Nachweis, dass intestinal appliziertes I^{125} markiertes *Salmonella* Flagellin in Ratten, bis zum Alter von 5 Tagen, antigen wirkt. Die Aufnahme des Flagellins im Ileum erfolgt über vakuoläre Zellen der Mucosa.

S. Koskimies[19] and J. J. Miller III[20]

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