could not be considered a suitable control group, they at least give some idea of distribution of these types of viruses in the population at the same time, for comparison by age group with the cholera patients.

There was no evidence that disturbance of the gastrointestinal tract by cholera increased virulence of the adenoviruses and enteroviruses isolated. All cholera patients from whom virus was isolated recovered promptly after adequate fluid and electrolyte replacement therapy, and no other illnesses were diagnosed during the convalescent period.

Summary. Virus isolation studies on rectal swab specimens of cholera patients were carried out in tissue cultures during the Bangkok, Thailand cholera epidemic of 1958 and 1959. An adenovirus or enterovirus was isolated from 6 of 44 patients with proven cholera. It was concluded that viruses which can be isolated in monkey kidney and HeLa cell tissue cultures were not involved in the cholera syndrome.

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Response of Peritoneal Exudate Cells to *Brucella melitensis.* Influence of Nature of Inflammatory Irritant.* (25814)

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For many years there has been increasing interest in the use of mononuclear cells from rabbit and guinea pig peritoneal exudates as a tool for study of specific and non-specific resistance to bacterial and viral infections. One difficulty in comparing findings of different inevstigators rests principally with use of different animal species as sources of the monocytes and with inoculation of different inflammatory agents into the peritoneal cavity. This subject has recently been reviewed (1). To learn whether the nature of the irritant influences activity of cells which accumulate in response to intraperitoneal inoculation into the rabbit, a comparison has been made in which such irritants as light mineral oil, sodium caseinate, dextran, glycogen and killed Brucella cells were used. Responses of monocytes from normal and Brucella-immune rabbits to parasitization by *Brucella melitensis* were studied by methods described(1).

Methods and materials. Irritants. "Klearol" (obtained from L. H. Butcher Co., San Francisco). Casein (supplied as sodium caseinate by Mann Assayed Biochemicals, approximately 90% protein). A 2.5% solution of the protein in saline was employed. Glycogen (Nutritional Biochemicals). Dextran (Cutter Labs) in the form of 6% solution for intravenous injection. *Br. melitensis,* scraped off Albimi agar slants after 3 days incubation at 37°C washed twice and resuspended in saline. Monocytes from adult rabbits, both normal and immunized, were injected with one of the inflammatory agents. For example, either sterile Klearol, 1.2% (W/V) solution of sodium caseinate, 6% Dextran (W/V), 0.1 mg % (W/V) glycogen or a formalin-killed, saline suspension (10^9/ml) of a virulent strain

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TABLE I. Responses of Peritoneal Exudate Cells to *Brucella melitensis*.

Stimulant	Type of cell	Cell counts 0	24	48	96	% degeneration 24	48	96
Oil	Normal, non-parasitized	195	190	194	267	3	0	0
	" parasitized	118	84	68	61	29	42	48
	Immune, non-parasitized	238	237	237	236	.5	0	.5
	" parasitized	432	430	432	431	.5	0	.3
Caseinate	Normal, non-parasitized	263	262	258	264	.4	2	0
	" parasitized	392	392	389	487	0	.8	0
	Immune, non-parasitized	350	349	353	310	.3	0	0
	" parasitized	82	100	100	100	0	0	0
Glycogen	Normal, non-parasitized	576	576	571	450	0	1	22
	" parasitized	373	370	371	300	1	0	20
	Immune, non-parasitized	155	160	168	123	0	0	27
	" parasitized	166	111	102	55	34	39	67
Dextran	Normal, non-parasitized	1062	1057	1040	1296	.5	2	0
	" parasitized	732	732	715	803	0	3	0
	Immune, non-parasitized							
	" parasitized							
Brucella melitensis	Normal, non-parasitized	442	438	444	438	1	0	1
	" parasitized	253	153	150	90	39	40	64
	Immune, non-parasitized	1349	1341	1330	1380	.6	1	0
	" parasitized	1320	1310	1325	1389	.8	0	0

of *B. melitensis* was injected intraperitoneally in 50 ml. Six days later monocytes were collected by washing peritoneal cavity with 200 ml Tyrode solution. The cell suspension was filtered through gauze and sedimented. Cells were redispersed in Tyrode solution containing 0.25 g % trypsin (Difco, 1:250). After 25 minutes at 25°C the cells were washed 3 times in chilled Tyrode solution. After a final centrifugation the packed cells were suspended in fresh homologous serum. Cells were parasitized by mixing a sufficiently concentrated saline suspension of a strain of *B. melitensis* with monocytes in paraffin-lined bottles. The mixtures were centrifuged, then held 1 hour at 4°C to allow 25 to 35% of monocytes to become infected. Supernatant fluid was then removed as completely as possible, sediment resuspended in small volume of medium consisting of 40% homologous rabbit serum and 60% Tyrode solution to contain approximately 15 monocytes/mm^3. Assembly and preparation of Mackaness cell culture chambers has been described(1). To obtain monocytes and sera from immune rabbits, the animals were inoculated subcutaneously 3 months earlier with 10^9 live cells of *B. melitensis* strain, Rev. I(2).

Results. Monocytes from normal as well as from Brucella-immune rabbits were parasitized with living, attenuated, vaccine strain of *B. melitensis*, Rev. I. Survival and maintenance of parasitized cells were recorded daily as described earlier(1). The results from 3 Mackaness chambers were gathered to measure response of each type of cell (Table I). Data are taken from representative experiments, of which 9 replicates were carried out in detail. Data from all 9 experiments were in excellent agreement, showing less than 0.5% variation. Table I shows markedly different responses of monocytes induced either by oil or *B. melitensis* suspension on the one hand, and casein, dextran and glycogen on the other. Responses of monocytes induced by oil or by killed *B. melitensis* were hardly distinguishable. Furthermore, monocytes of immune and normal animals responded to parasitization by living *B. melitensis* in a manner dependent upon immune or susceptible state of animal rather than upon the nature of inflammatory agent (oil or bacterial suspension).

In contrast, monocytes of normal rabbits appearing in response to casein and dextran behaved towards the parasitizing organism as if they came from immune rabbits, *i.e.*, monocytes induced by casein and dextran were not

destroyed by a strain of *B. melitensis* cytolytic for those monocytes induced by oil or bacterial suspension.

Glycogen on the other hand stimulated a paradoxical reaction in monocytes, in which the "immune" monocyte (in presence of homologous serum) was more susceptible to cytolytic action of the parasitizing bacillus than was the monocyte from non-immune animals. Table I also shows that glycogen-stimulated, parasitized monocytes from immune animals were destroyed more rapidly than parasitized monocytes from nonsensitized animals. Use of glycogen to induce peritoneal inflammation caused a strong tendency to peritoneal bleeding and monocytes taken from such animals were very often characterized by extreme fragility. When sufficient numbers of animals were used, it was possible to obtain a few in which such bleeding into the peritoneum did not occur; these animals yielded monocytes which appeared to be hypersensitive to cytolytic action of Brucella.

It was of interest to study survival values of mononuclear cells which appeared in response to various irritants. This quality is of particular importance in experiments on growth and multiplication of intracellular parasites and the variables affecting monocytic destruction of parasites(1).

To overcome the difficulty of removing monocytes from glass surfaces to which they had adhered, plasma clot was employed as the cultivating surface. For this purpose, blood was withdrawn from a rabbit and placed in a tube containing 0.1 mg of heparin/20 ml blood. Blood cells were immediately spun down and 0.5 ml of plasma was dispensed into several Carrel flasks. Simplastin (0.1 ml) was prepared by resuspending contents of 1 vial of commercial preparation (General Diagnostics Div., Warner-Chilcott Co.) in 8 ml distilled water. The clot formed very quickly after addition of Simplastin.

To each Carrel flask containing the plasma clot were added 1 x 10^4 mononuclear cells prepared as described above. Varying periods of time thereafter, duplicate flasks were treated with 0.25% trypsin for 30-60 minutes to remove the clot. A viable count of mononuclear cells was performed in a haemocytometer after cell suspension had been exposed to 0.001% (W/V) aqueous eosin.

Increases in cell population occurred for 6-9 days in the exudate induced by Klearol and glycogen: cells stimulated by dextran deteriorated under identical conditions within 72 hours.

Monocytes induced by oil were maintained under conditions of the Carrel flask plasma clot method for 4 months. This was done by treating the clot with trypsin, washing freed monocytes 3 times in Tyrode solution and replanting the cells onto the plasma clot at 10 day intervals.

Conclusion. Studies on mononuclear cell responses to *B. melitensis* require that attention be paid especially to the nature of inflammatory agent itself when interpreting behavior of the mononuclear cell. The inflammatory substance may so alter mononuclear response as to reverse cytolytic susceptibility of monocytes from normal and immunized animals.

Summary. A comparison has been made of the effect of several irritants on response of mononuclear cells from rabbit's inflamed peritoneum. Animals injected intraperitoneally with mineral oil or a suspension of killed *B. melitensis* stimulated accumulation of peritoneal monocytes whose behavior was identical towards living *B. melitensis, i.e.,* brucellae were cytolytic for normal rabbit monocytes but not for immune rabbit monocytes. Dextran and casein stimulated exudation of relatively insusceptible monocytes. Glycogen induced exudation of a relatively fragile monocyte, the susceptibility of which to Brucella parasitization was increased by the *immune state* of host animal.

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