TABLE I.
Effect of Vitamin E With and Without Feeding of Cholesterol on Cholesterol Content of the Blood, Aorta and Liver of Rabbits.

Experiment 3 times a week	No. of rabbits	Avg whole blood cholesterol (mg/100 ml) 0	2	4	8	12 weeks	Avg aorta cholesterol g/100 g dry wt	Avg liver cholesterol g/100 g wet wt
100 mg Vit. E intramuscularly	4	95	103	104	82	89	0.360	0.372
1 g cholesterol added to chow	6	101	123	194	329	325	0.897	0.743
100 mg Vit. E intramuscularly and 1 g cholesterol added to chow	12	103	105	285	392	448	2.165	0.794

serve this effect. The apparent discrepancy may be ascribed to the different routes and dosages employed in the two investigations. Dam fed vitamin E in concentrations of 100 mg of *d,l*-alpha-tocopherol per kg of oats-cholesterol mixture; the amount of this vitamin consumed by the rabbits in the experimental period of 85-97 days is not stated. Assuming a rabbit eats approximately 100 g of food per day, each rabbit in his series ingested about 900 mg of this vitamin in 13 weeks. In the present experiments, vitamin E was injected intramuscularly 3 times weekly for 12 weeks in doses of 100 mg per injection, *i.e.*, a total of 3600 mg of vitamin E per rabbit or about four times the amount of vitamin E probably used by Dam.

Conclusion. In cholesterol-fed rabbits, the intramuscular administration of *d,l*-alpha-tocopherol (vitamin E) in olive oil or sesame oil augments appreciably the deposition of cholesterol in the aorta but increases only slightly the concentration of this lipid in whole blood and liver.

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Inhibitory Effect of Glycogen upon Anaphylactic Shock in the Rabbit.

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In another paper from this laboratory[1] it was shown that hydatid fluid produces a sharp reduction in rabbit blood histamine and the leucocyte count. Since under urethane anesthesia, a similar decrease in blood histamine occurred, without leucopenia, the conclusion was drawn that both phenomena are independent. As platelets always disappeared from blood after the injection of hydatid fluid, we have concluded that the fall in blood histamine is more likely to depend on a decrease of platelets, additional evidence in favor of the theory proposed by Minard[2] and Zon, Ceder, and Crigler[3] that the main carriers of histamine in rabbit blood are the platelets. Submitting hydatid fluid to further fractionation, we have been able to verify that a macromolecular, polysaccharide fraction is the con-

* From the Butantan Institute, São Paulo, Brazil.

1 Graña, A., and Rocha e Silva, M., in press.

2 Minard, D., *Am. J. Physiol.*, 1941, **132**, 327.

3 Zon, L., Ceder, E. T., and Crigler, C. W., *Publ. Health Rep.*, 1939, **54**, 1978.

TABLE I.
Influence of Liver and Ascaris Glycogen and a Polysaccharide Fraction from Hydatid Fluid upon Blood Histamine and Leucocytes in the Rabbit.

Rabbit No.	Anesthesia used	Material injected	Blood histamine (γ/1 cc) Before	Blood histamine (γ/1 cc) After	W. B. C. ($\times 10^{-3}$) Before	W. B. C. ($\times 10^{-3}$) After	Effect upon blood pressure
1	None	Hyd. polys.	1.75	0.25	5.8	1.6	—
2	"	EE_0 (5cc)*	0.66	0.57	13.2	5.0	—
3	"	" "	2.30	0.23	8.6	1.6	—
4	"	Asc. glyc.	1.97	0.38	—	—	—
5	"	" "	1.80	0.23	13.8	4.2	—
6	"	L. glyc.	1.50	0.27	11.4	4.8	—
7	"	" "	1.40	0.42	—	—	—
8	Dial + ether	" "	1.75	0.81	29.2	3.6	No shock
9	" "	" "	1.10	0.62	12.4	6.2	" "
10	Chloralose	" "	1.50	0.28	6.2	6.6	" "
11	"	" "	1.60	0.23	6.4	10.6	" "
12	Urethane	EE_0 (5cc)	2.22	0.30	22.6	25.0	" "
13	"	L. glyc.	1.00	0.25	7.4	10.4	" "
14	"	EE_0 (5cc)	1.65	0.40	8.8	8.0	" "
15	"	Asc. glyc.	2.80	0.82	17.2	22.8	" "
16	"	Hyd. polys.	2.05	1.50	30.0	29.2	" "

* EE_0 means deproteinized and dialysed ascaris extract; Asc. glyc., represents the fraction obtained from EE_0 by precipitation with 50% ethyl alcohol; L. glyc. means a highly purified preparation from dog's liver glycogen; Hyd. polys. means the polysaccharide fraction prepared from hydatid fluid. The solid materials were usually injected in the dose of 100 mg in 5 cc of saline.

stituent responsible for this effect upon rabbit blood histamine and leucocyte-platelet count.

In the meantime, we have been studying the anaphylaxis-like reaction produced in dogs and guinea pigs by an Ascaris extract prepared according to Machebouef and Mandoul.[4] This Ascaris extract produces a shock in guinea pigs and dogs, that is indistinguishable from very severe anaphylactic shock. In the rabbit, this Ascaris material produces a sharp fall in blood histamine and leucopenia, but no shock. Concomitantly, the platelets disappear from blood. Under urethane anesthesia, however, there is rather a leucocytosis or no change in the leucocyte count, and yet the blood histamine is sharply reduced. Chemical fractionation of the Ascaris material showed that a glycogen-like material, which can be precipitated by 50% ethyl alcohol, is the constituent responsible for these effects upon blood histamine and the leucocyte-platelet count. This suggested the possibility that liver glycogen might produce a like effect, which is confirmed in the present paper.

Experimental. The rabbits were anesthetized with 1 g of urethane per kilo of body weight, by the intraperitoneal route. For Dial anesthesia, a dose of 0.50 to 0.65 cc of Dial "liquid" (Ciba) was injected with a complement by ether. For Chloralose anesthesia, a dose of 0.11 g per kilo was injected intravenously in 10 cc of warm saline. Duplicate samples (1 cc) of blood for histamine estimation were taken from the jugular vein in the anesthetized animals or from the ear vein or the heart in the unanesthetized animals, before and after the injection of the materials. The histamine extraction for estimation was made by Code's method[5] and the isolated guinea pig ileum used for the assay. For the anaphylaxis experiments, the animals were anesthetized with urethane or Dial + ether and the carotid blood pressure recorded. Sensitization to horse serum followed Grove's schedule.[6] The *Ascaris lumbricoides* were collected from hogs in the slaughter house and the extracts prepared according to Machebouef and Mandoul.[4] This extract (EE_0) produces an enormous emphysema in guinea pigs in doses up to 2 cc and a profound shock in dogs, when injected intravenously in doses up to 3 to 5 cc. The shock produced in guinea pigs

[4] Machebouef, M., et Mandoul, R., *C. R. Soc. Biol.*, 1939, **130**, 1032.

[5] Code, C. F., *J. Physiol.*, 1937, **89**, 257.

[6] Grove, E., *J. Immunol.*, 1932, **23**, 114.

and dogs has been studied in our laboratory and the results will be published elsewhere.[7] The extract EE_o contains a considerable amount of a glycogen-like material (positive iodine test), which can be precipitated by addition of an equal volume of absolute ethyl alcohol. This precipitate can be further purified by successive redissolutions and precipitations with 50% ethyl alcohol. The polysaccharide from hydatid fluid (*Toenia echinococcus*) was prepared by precipitating a 10 times concentrated, deproteinized and dialysed fluid, with 70% ethyl alcohol.

Results. Effect upon in vivo rabbit blood histamine. The experiments described in Table I were performed on 16 normal rabbits. Seven unanesthetized rabbits were intravenously injected with the indicated material and the blood histamine and leucocytes estimated before and after the injection. Microscopic slides were prepared for an evaluation of the presence of platelets. As a rule, the platelets were drastically reduced after the injection of the polysaccharide materials. Injection of 15 to 30 mg of glycogen per kilo of body weight is enough to remove 80 to 90% of the platelets previously present. The duration of this effect varies from 20 minutes to 1 hour, the platelets returning slowly to normal. Higher doses (100 to 200 mg) produce a more pronounced effect, for longer periods of time. There was considerable reduction in both the histamine content and the leucocyte count in every rabbit injected with polysaccharides extracted from Ascaris or hydatid fluid, as well as in those given 100 to 200 mg of purified glycogen. In another group of animals, 2 were anesthetized with dial + ether, 2 with chloralose and 5 others with urethane. As shown in Table I, in the animals submitted to Dial + ether anesthesia, there was the same drop in both the histamine and the leucocyte count, while in the rabbits submitted to chloralose or urethane anesthesia, there was rather a leucocytosis, although the histamine content fell sharply in all cases. In every case, the platelets fell sharply, almost to zero, after the injection of the polysaccharides. In a few animals, estimation of the platelet volume was made by using the thrombocytocrit, according to Van Allen.[8] In one rabbit submitted to urethane anesthesia, the platelet volume fell from 0.35/100 cc of blood, to 0.08/100 cc of blood, while the leucocytes varied only from 7,200 to 6,000. In another rabbit submitted to ether anesthesia, the platelet volume dropped from 0.60/100 cc of blood to 0.05/100 cc of blood, while the leucocytes varied from 10,600 to 1,200, after the injection of 300 mg of liver glycogen.

These findings are quite in agreement with those previously reported by us[1] with hydatid fluid and lend support to the theory that rabbit blood histamine is mostly carried by platelets. In perfect agreement with our previous experiments also, is the fact that this drop in the histamine content of rabbit blood can co-exist with no change in the carotid blood pressure (no shock), as shown in Table I. This definitely shows that the decrease in blood histamine observed in anaphylactic shock in the rabbit by Rose and Weil,[9] Katz,[10] and Dragstedt *et al.*[11] is not sufficient to explain the fall in carotid blood pressure which follows injection of the antigen into sensitized rabbits.

In vitro experiments with samples of rabbit blood. The addition of liver glycogen or the glycogen derived from Ascaris extracts to samples of rabbit blood do not produce a shift of histamine from cells to plasma, as occurs in anaphylactic,[10,11] trypsin,[12] and peptone shocks. This absence of histamine discharge from cells to plasma was also observed with hydatid fluid and appears to demonstrate that the production of an anaphylactic shock in the rabbit is rather concerned with a liberation of histamine from cells to plasma, as shown by Katz[10] and Dragstedt *et al.*[11]

Effect of liver glycogen upon the course of the anaphylactic shock in the rabbit. This extraordinary property of liver glycogen to

[7] Rocha e Silva, M., and Graña, A., in press.

[8] Van Allen, C. M., *J. Lab. and Clin. Med.*, 1926, **12**, 282.

[9] Rose, B., and Weil, P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 494.

[10] Katz, G., *Science*, 1940, **91**, 22.

[11] Dragstedt, C. A., Arellano, M. R., and Lawton, A. H., *Science*, 1940, **91**, 617.

[12] Dragstedt, C. A., and Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 420.

TABLE II.
Influence of Liver Glycogen upon the Course of Anaphylactic Shock in the Rabbit.

Rabbit No.	Anesthesia used	Material injected	Blood histamine (γ/1 cc) Before	Blood histamine (γ/1 cc) After	W. B. C. (× 10⁻³) Before	W. B. C. (× 10⁻³) After	Intensity of shock
17	Urethane	Glyc. + serum	1.70	0.64	17.0	4.0	±*
18	"	" "	1.40	0.50	—	—	0
19	Dial + ether	" "	1.85	0.33	12.0	2.0	+
20	" "	" (3 g)	1.40	0.07	14.6	4.0	0
"	Same (7′ after)	Serum (4 cc)	0.07	0.12	4.0	2.6	0
21	Dial + ether	Glyc. (3 g)	2.70	0.50	9.1	4.1	0
"	Same (9′ after)	Serum (4 cc)	0.50	0.27	4.1	1.5	0
22	Urethane	Glyc. (0.5 g)	1.35	1.00	9.3	7.5	0
"	Same (2′ after)	Serum (4 cc)	1.00	0.27	7.5	2.6	++
23	Dial + ether	Glyc. (0.5 g)	2.40	2.00	10.9	8.4	0
"	Same (50″ after)	Serum (5 cc)	2.00	0.63	8.4	1.7	+++
24	Dial + ether	Serum (5 cc)	2.40	1.50	9.8	2.8	++
"	Same (2′ after)	Glyc. (0.5 g)	1.50	0.82	2.8	1.7	+++
25	Dial + ether	Serum + glyc.	1.68	0.20	11.6	3.2	++
26	" "	" "	2.15	0.73	14.4	7.2	+++

+++, rapidly fatal shock; ++, long standing fall in carotid blood pressure, recovery in 15 to 30 minutes; +, evanescent fall in carotid blood pressure, return to normal in 5 to 10 minutes; ± or 0, slight or absent shock.

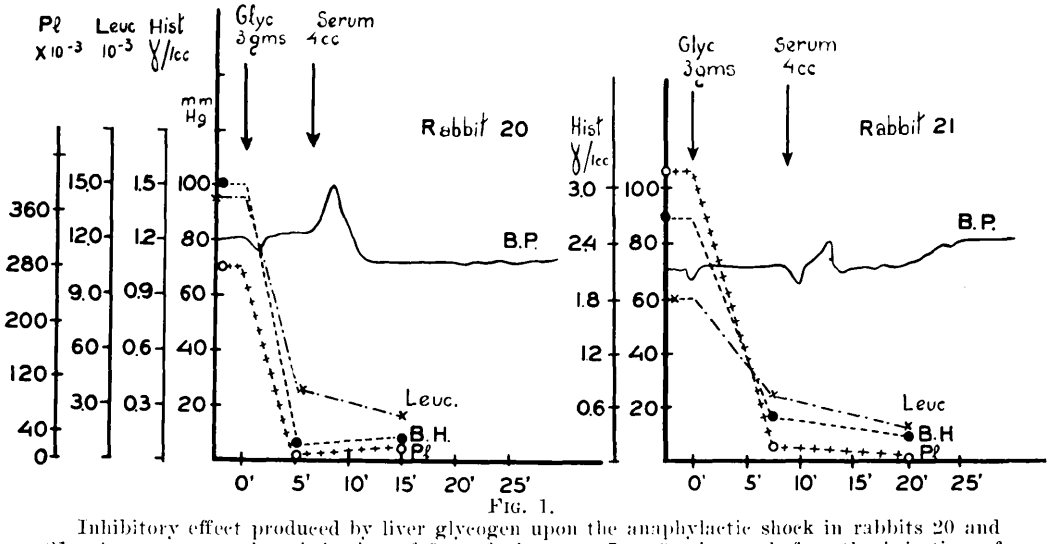

FIG. 1.

Inhibitory effect produced by liver glycogen upon the anaphylactic shock in rabbits 20 and 21. As seen, a previous injection of 3 g of glycogen, 7 to 9 minutes before the injection of serum, produces drastic reductions in blood histamine and the leucocyte-platelet count and prevents the drop in carotid blood pressure, which usually follows the injection of serum in sensitized rabbits.

drastically reduce the histamine from rabbit blood without producing shock or a discharge of histamine from blood elements to plasma, appeared to provide a means to study further the mechanism of anaphylactic shock in the rabbit. This always appeared to be the consequence of a clumping of leucocytes[14] and probably also of platelets in the small vessels and capillaries of the lung, and of a discharge which aggravates the picture, by constricting the pulmonary vessels.[11] Our experiments showing that a considerable reduction in leu-

[13] Gotzl, F. R., and Dragstedt, C. A., *J. Pharm. and Exp. Therap.*, 1942, **74**, 33.

[14] Abell, R. G., and Schenck, H. P., *J. Immunol.*, 1938, **34**, 195.

cocytes and platelets is not enough to produce shock seem to warrant the idea that the liberation of histamine constitutes a very important factor in localizing to the lung capillaries the emboli produced by the clumping of blood elements.

In 6 rabbits we have studied the effects of a previous injection of glycogen upon the course of anaphylactic shock and in 5 others, glycogen was given after the injection of the antigen. It became apparent (Table II and Fig. 1) that if a proper amount of glycogen is injected (1 to 3 g) and enough time is allowed to elapse (5 to 10 minutes) before injection of the serum, the blood histamine, the leucocytes, and the platelets are drastically reduced and shock will not occur after the injection of serum. This is clearly shown in rabbits 20 and 21. When an insufficient amount of glycogen is injected 1 to 2 minutes before the serum, the shock which occurs is either milder or unchanged. Given after the serum, glycogen rather aggravated the condition or had no effect at all.

Interesting enough is the fact that the leucopenia in anaphylactic shock probably derives from a different mechanism than that produced by glycogen, since under urethane anesthesia, glycogen produces a leucocytosis or no change in the leucocyte count, while anaphylactic shock produces a drop in leucocytes even under these anesthetics. Since histamine is bound to platelets, it seems logical to conclude that the reduction in blood histamine during anaphylactic shock in the rabbit depends upon the removal of the platelets from circulating blood, this being an inevitable occurrence during this kind of shock.[15]

Summary. Polysaccharides prepared from *Ascaris lumbricoides* and hydatid fluid, as well as liver glycogen, produce a sharp reduction in blood histamine in the unanesthetized rabbit or in those under Dial + ether anesthesia, urethane and chloralose anesthesia. Concomitantly, platelets almost disappear from circulating blood while the leucocytes are reduced only in the unanesthetized rabbit or in those under Dial + ether anesthesia. Under chloralose or urethane anesthesia, there was rather a leucocytosis after the injection of glycogen. Thus the conclusion was drawn that histamine is bound to platelets in rabbit blood. Glycogen has a definite inhibitory effect upon the course of anaphylactic shock, explained by the fact that glycogen *disperses* the blood elements, without a preferential allocation of those in the shock organ, while anaphylactic shock in the rabbit occurs as a consequence of the clumping of leucocytes and platelets in the lung capillaries and small vessels, which become a filter for agglutinated blood elements.

[15] Kopeloff, N., and Kopeloff, L. M., *J. Immunol.*, 1941, **40**, 471.

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Alloxan in Pregnant Rats.*

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Diabetes mellitus can be produced in rats by the injection of alloxan.[1] Although there is slight damage to other tissues of the body, the principal action of alloxan is the production of a selective necrosis of the beta cells of the islets of Langerhans. The diabetic state produced can be ameliorated by adrenalectomy[2] and by insulin therapy.[3]

In an attempt to produce offspring with

* This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **245**, 384.

[2] Janes, R. G., and Friedgood, C. E., *Endocrinology*, 1945, **36**, 62.

[3] Kaplan, N. O., Franks, M., and Friedgood, C. E., *Science*, 1945, in press.