survival time to implantation time of second set skin homografts in the rat was studied. 2. Second set homografts implanted simultaneously with, or 5 days after the first set graft, develop an active hemal circulation and are rejected simultaneously with first set graft. 3. Second set homografts implanted 6 or 7 days after first set graft never become revascularized and are considered to survive for zero days. 4. Second set homografts implanted 8-30 days after first set graft develop an active hemal circulation and survive for a mean time of 5.1 days. 5. Second set homografts implanted 60 days after first set graft show a normal first set survival time of 8 days. 6. There is no significant difference in survival time of second and third set grafts when third set is implanted 7 days after the second set graft. 7. It is concluded that temporal sequence in accelerated breakdown of second set skin homografts is suggestive of an immunological mechanism triggered by the host's contact with the first set graft. This is in agreement with Medawar's theory of acquired immunity.

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Biosynthesis of Ascorbic Acid and Prevention of Glycogen Depletion in Liver and Muscle. (21253)

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We previously reported that gradual accumulation of fat metabolites (like acetoacetate and β-hydroxybutyrate in the systems of rabbits and guinea pigs) is responsible not only for the onset of hyperglycemia following a hypoglycemia but also for causing decreased glucose tolerance, depletion of reduced glutathione of blood and other diabetic symptoms (1-6). Tidwell and Nagler(7) observed that fasting blood sugar level of rats were significantly depressed by repeated daily injections of acetoacetate, without showing its eventual rise as in rabbits. They referred to the work of Parnes and Wertheimer(8) to explain such effect on blood sugar levels of rats injected with acetoacetate on the basis of decreased glycogenolysis or increased glycogenesis. We have failed to coroborate such observations. They reported, on the other hand, that acetoacetate markedly increased glycogenolysis(9). That acetoacetate depresses glycogenesis is also evident from the observation of Chari and Wertheimer(10) that acetoacetate has a specific effect on glycogen synthesis. Tidwell and Nagler(7) also referred to our work(11) stating that acetoacetate markedly depletes glycogen storage in liver and muscle of rats. This statement also seems inaccurate. Most of our work was done on rabbits. We never reported that sodium acetoacetate depletes liver and muscle glycogen of rats. It is likely that species difference might play an import-

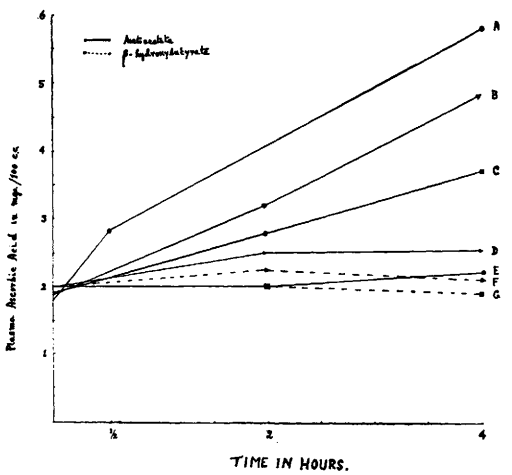

FIG. 1. Biosynthesis of ascorbic acid in rats after acetoacetate and β-hydroxybutyrate injections.

Doses in mg/kg body wt:

A	180	C	300	E	600	G	500
B	200	D	500	F	50		

ant part in response to diabetogenic substances.

Ascorbic acid is highly beneficial in improving glycogen synthesis(12) as well as ketolysis(13). According to Mosonyi(14) animals which can synthesize ascorbic acid react to administration of chemicals, especially some ketones by increasing their ascorbic acid excretion. Attempts, therefore, were made to see if such difference in behavior between rats and rabbits induced by acetoacetate could be accounted for through difference in capacity for immediate biosynthesis of ascorbic acid caused through injection of "acetone bodies" such as acetoacetate and β-hydroxybutyrate.

Experimental. Twenty-four healthy albino rats each weighing approximately 100-150 g and 16 rabbits each weighing approximately 2 kg were selected. Rats were maintained on the laboratory stock diet. Rabbits were given soaked Bengal gram (*Cicer arietinum*). Sodium acetoacetate and β-hydroxybutyrate were injected intramuscularly in doses shown in Fig. 1. Blood was collected from the marginal ear vein of rabbits. As the rats did not give sufficient blood for ascorbic acid estimation, equal quantities of blood from tails of 2 animals were mixed. Ascorbic acid was determined colorimetrically with indophenol dye according to the method of Bessey(15). Results are shown graphically in Fig. 1. Results with rabbits are represented in Fig. 2.

Since acetoacetate in higher concentration depresses biosynthesis of ascorbic acid in rats (Fig. 1), we investigated whether such high doses of acetoacetate would deplete glycogen storage of their liver and muscle. Twenty-six young rats weighing 100-150 g were fed the same diet as in the previous experiment(11). Ten animals were given daily injection of 500 mg/kg body weight of sodium acetoacetate, and 8 animals given dose of 200 mg/kg body weight. The animals were killed at intervals and glycogen in liver and muscle estimated according to the method of Good, Krammer, and Somogyi(16). Blood sugar was estimated according to the method of Hagedorn and Jansen(17). The results are shown in Table I.

In order to see the cumulative effect of sodium acetoacetate and β-hydroxybutyrate on blood ascorbic acid, 4 rats were injected daily with 200 mg/kg body weight of acetoacetate and 4 rats with β-hydroxybutyrate in daily dose of 50 mg/kg body weight. Animals were killed by decapitation after 55 days injection and plasma ascorbic acid estimated according to the method of Bessey(15). Results are shown in Table II.

Results. The results recorded in Figs. 1 and 2 illustrate that although rabbits are unable to synthesize ascorbic acid when injected

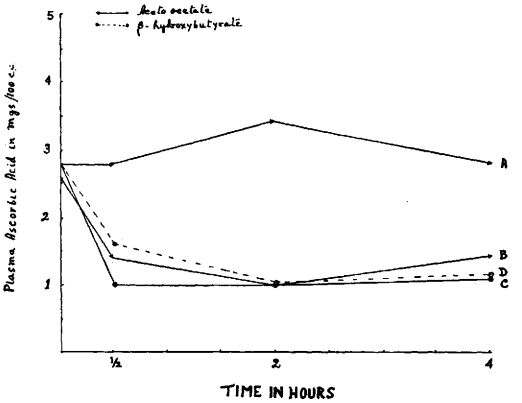

FIG. 2. Biosynthesis of ascorbic acid in rabbits after acetoacetate and β-hydroxybutyrate injections. Doses in mg/kg body wt:

A	60	B	180	C	500	D	500

TABLE I. Effect of Sodium Acetoacetate on Blood Sugar and Glycogen Level of Liver and Muscle in Rats. (Animals were killed after 18 hr fasting.)

No. of animals	Dose of aceto-acetate	Period of inj. (days)	Glycogen, mg/100 g tissue — Liver	Glycogen, mg/100 g tissue — Muscle	Blood sugar in mg/100 cc
4	0	0	260 ± 8.2	128 ± 10.2	82 ± 1.5
2	200	10	250 ± 10.2	140 ± 6.1	78 ± 2.5
2		25	255 ± 8.8	128 ± 8.2	70 ± 3.8
2		40	248 ± 5.8	120 ± 10.2	72 ± 4.2
2		55	240 ± 6.2	130 ± 10.8	72 ± 4.3
2	500	10	202.2 ± 15.2	160 ± 12.2	65.0 ± 1.2
3		30	140.6 ± 7.8	47.3 ± 5.8	140.0 ± 8.2
3		55	88.6 ± 6.5	25.3 ± 3.2	190.0 ± 10.2
2*		69	—	—	210 ± 8.2

* The animals showed glycosuria (.36%) on 70th day.

TABLE II. Effect of Repeated Daily Injection of Acetoacetate and β-hydroxybutyrate (Sodium Salt) on Plasma Ascorbic Acid.

No. of animals	Substance injected	Dose, mg/kg	Period of inj. (days)	Plasma ascorbic acid, mg/100 cc
4	0	0	0	2.08 ± .12
4	Acetoacetate	200	55	1.98 ± .20
3		500	55	.88 ± .02
4	β-hydroxybutyrate	50	55	.98 ± .04

with acetoacetate, beyond a certain concentration, rats are capable of doing so depending inversely on concentration of acetoacetate. With concentrations as high as 600 mg/kg there was a definite depression in the process. β-hydroxybutyrate showed, however, no effect on biosynthesis of ascorbic acid either in rats or rabbits.

It is also evident (Table I) that acetoacetate in very high concentration can deplete liver and muscle glycogen even in rats and can bring about a marked increase in blood sugar concentration on repeated injection for a number of weeks. Tidwell and Nagler(18) began with a comparatively low concentration of acetoacetate and could not observe any such effect. Our work with lower concentration of acetoacetate also shows similar result (Table I). The failure of acetoacetate in depleting liver and muscle glycogen of rats when injected in such low concentration may be attributed to the biosynthesis of ascorbic acid which has been found to be stimulated by acetoacetate up to a certain concentration.

Summary. 1. Injection of sodium acetoacetate, in low concentrations (up to 200 mg/kg) stimulated biosynthesis of ascorbic acid in rats and did not disturb carbohydrate metabolism by raising blood sugar level. 2. This compound at higher concentration (600 mg/kg), however, depressed such biosynthesis, raised blood sugar and depleted liver and muscle glycogen in rats. 3. Sodium β-hydroxybutyrate depressed biosynthesis of ascorbic acid in rats. 4. Rabbits failed to synthesize ascorbic acid after acetoacetate or β-hydroxybutyrate injection. 5. Depression in biosynthesis of ascorbic acid in animals is shown to be associated with disturbance in carbohydrate metabolism.

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Demonstration of Anatomy of the Giant Fiber System of the Squid by Microinjection.* (21254)

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There are a number of morphological, physiological and pharmacological technics available for the study of the gross and microscopical features of the nervous system. In this paper is described still another technic, that of microinjection which has been adapted to demonstrate the finer structures at the cellular and intercellular levels. With this technic, single neurons can be colored and their exact courses, branches and connection traced.

Materials and methods. The squid (*Loligo pealii*) was used, the gross aspects of the giant fiber system having been demonstrated by Young(1). If only the mantle fibers and the stellate ganglion were needed, the animal was decapitated; if the central nervous system was needed intact, the animals were narcotized with chloral hydrate (1 g/liter filtered sea water). The mantle was opened by a mid-ventral incision, flattened against a glass plate, and firmly anchored (Fig. 1). The specimen was kept wet with sea water during its use. It was not necessary to remove the fibers from the mantle because of the latter's translucence. However, when the second order fiber from the stellate ganglion into the central nervous system was to be injected, it had to be dissected free from the other fibers making up the bundle. A binocular dissecting microscope was adequate. The specimen on a glass plate was lighted from below. A simple right-angle coarse-geared micromanipulator was used. Micropipettes with tips 5-10 μ were hand drawn from 0.85 mm bore hard glass tubing, according to the method of Barber as modified and extensively used by Chambers(2,3). Pipettes were filled just before use with 0.1-0.5% Trypan Blue or 1% Trypan Red(4). Under the dissecting microscope the microtip

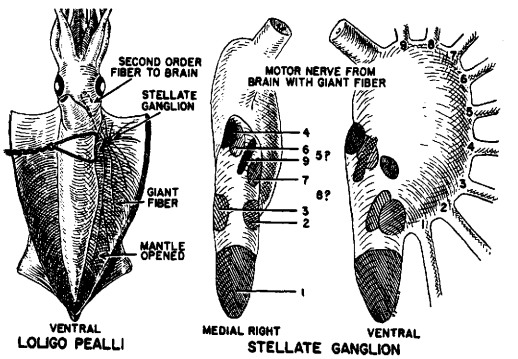

FIG. 1. The giant fibers of the mantle decrease in size from posterior to anterior so that fiber 1 is huge compared with 8 or 9. Nucleus 1—Several injections. This is the largest cell mass because it forms the largest fiber. It occupies the entire posterior of the ganglion's cell mass. Nucleus 2—Several injections. This mass is somewhat smaller, since its fiber is smaller. It lies immediately anterior to 1, but occupies only the ventral portion of the total mass. Nucleus 3—Three injections. A smaller elongated group of cells lying dorsal to 2. Nucleus 4—One injection. A small cell group at the antero-dorsal tip of the extraganglionic cell mass. Nucleus 5—No satisfactory injection. One attempt. Nucleus 6—One injection. About the same size as 4 and lying just medial to it. Nucleus 7—One injection. A small group just anterior to 2 on ventral side of the whole cell mass. Nucleus 8—No satisfactory injection. Three attempts. Nucleus 9—One injection. A very small elongated group of cells lying ventral to 6.

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