The K_m value for mesotartaric acid in this system is high, $1.4 \times 10^{-2}M$, compared with the K_m value for DL-malate, $6.2 \times 10^{-3}M$. The K_m value found for DPN was approximately $5 \times 10^{-3}M$. None of the carboxylic acids tested as potential inhibitors of DL-malate oxidation were found to inhibit mesotartaric acid oxidation in pigeon-liver extracts.

Similarly, addition of mesotartaric acid to rat-liver mitochondria resulted in an increased rate of oxygen uptake but much lower activities were found with rat-kidney mitochondria.

DISCUSSION

Sodium DL-malate is oxidized anaerobically by ferricyanide in the presence of pigeon-liver extracts. The oxidation is not catalysed by the malic enzyme since it could be shown that in these extracts TPN is essential for malic enzyme activity, whereas no coenzyme requirement for anaerobic oxidation by ferricyanide could be demonstrated. Further, the marked inhibition of this oxidation by tartronic and maleic acids and the much lower K_m value are not in agreement with the properties of the malic dehydrogenase of pig-heart extracts or resting suspensions of *Esch. coli*. It seems most likely that the responsible enzyme is similar to malic dehydrogenase but with physical constants differing from those found for the enzyme obtained from other sources. A binding of coenzyme by the dehydrogenase similar to that of DPN by glyceraldehyde 3-phosphate dehydrogenase (Velick, Hayes & Harting, 1953) may occur, however, and thus obviate the need for additional coenzyme.

This dehydrogenase is inhibited by mesotartaric, tartronic, maleic, malonic, isocitric and citric acids, and the inhibition by mesotartaric, tartronic and citric acids has been shown to be competitive. The K_i values found are not in agreement with those reported for L-malic dehydrogenase or the malic enzyme of various origins.

mesoTartaric acid is oxidized in pigeon-liver extract by a dehydrogenase which requires the presence of either DPN or TPN. With DL-malate or mesotartaric acid oxidation, the products do not seem to inhibit the reaction to a great extent since, in general, the initial velocities are maintained. In contrast, little oxidation of lactate or isocitrate could be obtained in the absence of a fixative. The dehydrogenase oxidizing mesotartaric acid is not the same as that oxidizing malate since it has a demonstrable DPN or TPN requirement and is not inhibited by any of the compounds shown to inhibit malate oxidation in the same system.

SUMMARY

The kinetics of a characteristic malic dehydrogenase of pigeon-liver extract have been investigated and a DPN-linked dehydrogenase oxidizing mesotartaric acid in the same system is described.

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Antigenicity of Insulin: Diabetes Induced by Specific Antibodies

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Lewis (1937) sensitized guinea pigs with ox or pig insulin. Using the Dale technique, he observed contractions of uterine strips from these animals in response to both ox and pig insulin. There was a more persistent response to the preparation of insulin which had been used to sensitize the animal. Bernstein, Kirsner & Turner (1938) have reported anaphylaxis in guinea pigs sensitized and challenged

with various insulin preparations. Wasserman & Mirsky (1942) showed that anaphylaxis could be induced in guinea pigs with insulin of a different species than that used for sensitization. Banting, Franks & Gairns (1938) reported the development of resistance to insulin in a non-diabetic schizophrenic patient following a course of insulin shock treatment. Blood serum from this patient, mixed with

insulin and injected into mice, gave a lower incidence of convulsions than did corresponding control mixtures. Lowell (1944) obtained similar evidence for neutralization of insulin with blood sera from two diabetic patients who were resistant to insulin. There is, however, the opinion that insulin is not an antigen. (Haurowitz, 1950; Sherrill & Lawrence, 1950.) In the work which is reported here, it is established that insulin can exercise a true antigenic effect as manifested by anaphylaxis and by the production of neutralizing antibodies.

MATERIALS AND METHODS

Preparation of antigen. Crystalline ox and pig insulin were used for sensitization. The insulin was injected subcutaneously in the form of a stable water-in-oil emulsion, using Freund's adjuvant (Freund & McDermott, 1942). The emulsion was prepared by adding a solution of insulin, 1 mg./ml. (25 units), pH 2.5–3.0 with HCl and containing phenol (0.3%, w/v), to an equal volume of a mixture (9:1, by vol.) of a light paraffin oil (Bayol F, b.p. range 274–380°, Imperial Oil Co.) and mannide mono-oleate (Arlacel A, Atlas Powder Co.). Emulsification was effected by rapid mechanical stirring, using a Waring Blendor or Virtis homogenizer. It is essential that a stable emulsion be obtained, in order to avoid severe hypoglycaemic reactions in the injected animals.

Preparation of crude insulin. Crude insulin extracts were prepared according to the first steps of a method described by Romans (1954). To each 100 g. of minced fresh pancreas were added 250 ml. of 92% (v/v) ethanol and 20 g. of NaCl. The mixture was stirred for 1 hr., during which time the pH was maintained at 5.0–5.2 by the addition of HCl. Solids were then separated by centrifuging and again extracted for 1 hr. with 125 ml. of 70% (v/v) ethanol. The mixture was centrifuged and the two supernatants were combined. Each 100 ml. of the combined supernatants was diluted with 160 ml. of water and shaken in a separating funnel with 25 ml. of CCl_4 . The CCl_4 layer was discarded and the aqueous layer dialysed in cellophan sacks against water acidified with HCl to pH 2.5–3.0. The crude insulin extracts of pig, ox, sheep and rabbit at this stage were sufficiently potent for our experimental work. Because of the lower yield (less than 0.5 unit/g. guinea pig pancreas), the dialysed extracts of guinea pig insulin were usually concentrated.

Inoculation of guinea pigs. Each guinea pig was given subcutaneously 1 ml. of antigen into each of two separate areas (a total of 1 mg. of crystalline insulin). This dose was repeated 4 weeks later and at other intervals as needed. Anaphylaxis and neutralizing antibodies can be demonstrated in some inoculated guinea pigs 4 weeks after a single injection of antigen. However, a better response both in respect of anaphylaxis and of neutralizing antibodies is obtained 2 weeks after a second or subsequent injection of antigen.

Bleeding and injecting. Guinea pigs were bled by cardiac puncture and injected intracardially or intravenously. Blood samples were taken from mice by a modification of a method described by Kassel & Levitan (1953). The mouse was held in the left hand of one operator, ventral surface uppermost; the left fore paw was held in the right hand.

A piece of cotton gauze attached to a weight of about 50 g. was caught on the incisors and allowed to weigh down the head. A second operator exposed the left external jugular vein using fine scissors and forceps. Blood was drawn with a syringe (0.25 ml.) fitted with a 26-gauge needle; immediately before use the syringe was rinsed with a solution of heparin (1000 units/ml.). The needle was inserted into the vein through the adjoining thoracic muscle in order to avoid tearing the vein. By this technique, four samples (0.1 ml. each) of blood have been taken within 3 hr., without apparent harm to the animal. Injections of 0.5–1.0 ml. of liquid can also conveniently be made into the jugular vein.

Blood-sugar determinations. Samples, obtained as described above, were transferred from the syringe to a 0.10 ml. blood capillary pipette. Blood-sugar estimations were done by the method of Somogyi (1952) with Nelson's (1944) arsenomolybdate reagent.

Acetone bodies. Urine was collected in test tubes from individual mice kept in glass funnels covered with cheese cloth. Acetone bodies were detected in 0.25–1.0 ml. of urine by the method of Legal as modified by Jackson-Taylor (Gradwohl, 1943).

Buffered saline. Phosphate buffer (0.15 M- Na_2HPO_4 -HCl, pH 7.4), was combined with an equal volume of 0.9% (w/v) NaCl.

RESULTS

Anaphylaxis in guinea pigs. Guinea pigs were sensitized with crystalline pig or ox insulin according to the method described. Anaphylaxis was readily induced in these animals by intravenous or intracardiac injection of 10–25 units of insulin. For example, of twelve guinea pigs which had received injections of pig insulin as antigen, eight exhibited symptoms of severe anaphylactic shock following an intracardiac injection of 12 units of insulin into each animal. With sensitized animals the symptoms of anaphylaxis including death appeared within 5 min. of the challenging injection, whereas with normal control animals, no symptoms of hypoglycaemic shock were observed within 3 hr. of the injection of corresponding amounts of the same insulin solution, and there were no deaths. It was found that anaphylactic shock could be induced by the injection of pig or ox or sheep insulin into animals sensitized by either pig or ox insulin.

Neutralization of insulin by immune serum from guinea pigs. The neutralizing activity of serum from guinea pigs which had been inoculated with crystalline pig or ox insulin was tested by mouse-convulsion assay procedure. Tests were carried out by injecting starved mice with serum or dilutions of serum mixed with an amount of insulin which, when injected in saline or normal serum, caused convulsions in 95–100% of the mice. The results of a typical test are shown in Table 1 and indicate that 1 ml. of immune serum can neutralize about 1 unit of insulin. The most potent sera which we obtained have shown a neutralizing activity of 4 units of insulin/ml. Sera of the above type, prepared by the

inoculation of crystalline pig or ox insulin, neutralized to the same extent ox insulin and pig insulin. Using the mouse-convulsion technique, it was found that these sera neutralized not only crystalline pig and ox insulin, but also crude pig, ox, sheep and rabbit insulins. No neutralizing activity was ever observed with normal guinea pig, rabbit or sheep sera. The following are typical results. Two groups of twelve mice were injected subcutaneously with mixtures of sheep insulin and serum. In one group the serum was from normal guinea pigs and in the other the serum was obtained from guinea pigs inoculated with crystalline pig insulin. Eleven of the twelve mice in the first group went into convulsions, whereas none of the twelve mice in the second group did. An experiment with rabbit insulin gave similar results. In this experiment normal guinea pig serum was replaced with saline. Six mice which received rabbit insulin and immune serum did not go into convulsions whereas six mice receiving insulin-saline did.

Six batches of guinea pig insulin were prepared. Five of these could not be neutralized by immune serum but one could. This batch was prepared from glands which had been stored in the cold for 6 months. All the other batches were prepared from fresh glands. It must be admitted, nevertheless, that we do not know the conditions necessary for the preparation of the type of guinea pig insulin which can be neutralized by specific serum. The words native and altered will be used to designate non-neutralizable and neutralizable guinea pig insulins respectively.

Neutralization of insulin by immune serum was demonstrated not only by the mouse-convulsion technique but also by changes in blood-sugar levels of mice injected with immune serum and insulin.

Some results are shown in Table 2. In the group of mice which had received saline, the injection of pig insulin effected a marked lowering of blood-glucose levels. In the mice which had received immune serum and then pig insulin, glucose levels continued to rise. In the mice which had received immune serum followed by native guinea pig insulin, blood-glucose levels were lower after 2 hr. than after 1 hr. It should be noted that in the group of mice which received native guinea pig insulin, only half as much insulin was injected as in the groups which received pig insulin.

Table 1. *Prevention by immune serum of convulsions due to insulin*

The pooled serum from twelve guinea pigs was used. The animals had been given two injections of crystalline pig insulin with an interval of 4 weeks between injections. Two weeks after the second injection, sera were collected and pooled. The insulin which was used for the test of neutralization was a solution of pig insulin, 1.2 units/ml., pH 2.7 with HCl. Serum, or serum diluted in buffered saline pH 7.4 (1 ml.) was combined with 0.2 ml. of insulin solution; the pH of the mixtures was in the range 6.9-7.4. Separate groups of four mice were used for each mixture. The mice were injected subcutaneously with 0.25 ml. per animal. The number of mice showing convulsions was observed as in the method for insulin assay.

Serum in 1.2 ml. of mixture containing 0.24 unit of insulin (ml.)	No. of mice showing convulsions in a group of 4
1.0	0
0.5	0
0.25	0
0.125	4
0.063	4
0.031	4

Table 2. *Changes of blood-sugar levels in mice following injections of immune serum and insulin*

Mice were divided into three groups of four, and a sample of blood was taken from each mouse. The animals of one group were then each injected intravenously with 0.5 ml. of saline, the animals of the other two groups with 0.5 ml. each of serum from guinea pigs immunized with crystalline pig insulin. One hr. later, blood samples were again taken. Immediately after each mouse was bled it was injected with insulin (0.25 ml.). One hr. after the injection of the insulin, samples of blood were taken for the third time.

Blood glucose (mg./100 ml.)					
Mouse	Before any injection	First injection	1 hr. after saline or immune serum	Source of insulin injected and dose (units)	1 hr. after insulin injection
1	95	Saline	115	Pig, 0.05	72
2	75		75		14
3	88		97		26
4	110		125		39
5	76	Immune serum	190	Pig, 0.05	260
6	110		215		260
7	78		202		230
8	100		222		260
9	120	Immune serum	235	Native guinea pig, 0.025	155
10	82		195		125
11	81		201		182
12	100		210		129

In other experiments with mice it was shown that immune serum neutralized pig insulin injected 10 min. before the immune serum as indicated by preventing convulsions and reversing the fall in blood-glucose levels. On the other hand, immune serum was ineffective in preventing the continued lowering of blood-glucose levels when native guinea pig insulin was injected 10 min. before the immune serum.

One is justified in ascribing the neutralizing activity of these sera to antibodies which have developed in response to injections of insulin for the following reasons: (1) The guinea pigs which produced the serum were susceptible to anaphylactic shock induced by insulin. (2) The neutralizing activity of the serum was non-dialyzable and was present in the fraction of serum precipitated by 50% and not by 30% saturation with ammonium sulphate. (3) It was possible to release insulin neutralized by serum. This was shown in the following experiment. Serum and insulin were combined at neutral pH, and left overnight in a cold room. The combined serum and insulin was then divided into two equal portions. One portion was acidified to pH 2.3 (3 drops of M-HCl/ml.) and left at room temperature for 1 hr. The acidified portion caused convulsions in all four mice injected. The untreated portion caused no convulsions in the four mice injected.

Diabetes induced in mice by immune guinea pig serum. It is shown in Table 2 that the injection of immune serum into mice was followed by a rise in blood-glucose levels. Results which bear directly on this question are given in Table 3. The glucose concentrations of the immune and normal sera in this experiment were substantially equal, namely 79 and 81 mg./100 ml. respectively. With the immune serum group, the blood-glucose levels at 3.5 and at 21 hr. were markedly elevated; 140 hr. after the final injection of immune serum, blood-glucose levels were comparable to those at zero time.

When injections of immune serum were administered daily for 3 days and tests were carried out on the second, third and fourth days, it was found that blood-glucose levels were elevated as shown in Table 4. There was a suggestion that the injections of immune serum caused a decrease in body weight. The results recorded for mice 12 to 17 were obtained in a later experiment. The pooled urine of mice 12 to 15 on the third day gave a strongly positive test for acetone bodies but the test on the urine from the normal serum group was negative. Tests for the presence of acetone bodies were carried out on the urine of each of seven mice which were given daily injections of immune serum as described in Table 4. On the second day all the tests were negative; on the third day, the tests were positive for three, but negative for four, mice. In this experiment the weights of the mice on the first day were 30, 28, 31, 30, 33, 31 and 30 g. and the losses in weight by the third day were 5, 5, 6, 6, 6, 8 and 6 g. respectively. These weight changes in response to immune serum are in accord with the results in Table 4.

Neutralization of insulin by immune serum from a sheep. A sheep was given a series of subcutaneous injections of crystalline pig insulin in Freund's adjuvant over a period of 3 months without producing demonstrable antibodies, as indicated by the mouse-convulsion technique. After a period of 10 months without treatment, an injection of insulin was again given. Two weeks later the serum of the sheep contained neutralizing antibodies. This serum was found to neutralize insulin of the following species: crystalline pig, crystalline ox, crude rabbit, crude and crystalline sheep, and the crude guinea pig insulin preparation which was neutralized by immune guinea pig serum. The immune sheep serum did not neutralize the type of crude guinea pig insulin which was not neutralized by immune guinea pig serum.

Experiments on the effect of immune sheep serum on blood-glucose levels in mice were undertaken but

Table 3. *Elevation of blood-glucose levels in mice due to injection of immune serum*

Blood samples were taken from each of 6 mice which had been starved for 4 hr. Three of the mice were injected with normal guinea pig serum and 3 with immune serum (neutralizing value 1 unit of insulin/ml.). Injections (0.5 ml.) were given to each mouse at zero time (intravenously) and at 1.5, 3.0 and 4.0 hr. intraperitoneally. Blood samples were again taken at 0.5, 3.5, 21 and 144 hr.

Mouse	Blood glucose (mg./100 ml.)				
	0 hr.	0.5 hr.	3.5 hr.	21 hr.	144 hr.
Normal serum					
1	65	76	68	92	93
2	71	75	64	98	87
3	70	114	84	121	100
Immune serum					
4	43	91	167	284	58
5	92	180	285	334	Dead
6	61	98	281	304	51

proved inconclusive. The following experiment throws some light on this problem. Four mice were injected with a mixture of equal volumes of normal and immune guinea pig serum. Four other mice were injected with a mixture of equal volumes of normal sheep serum and immune guinea pig serum. The mice in the first group showed a typical rise in blood-glucose levels; the mice in the second group showed no rise, but rather a slight drop below normal. It may be that this effect of sheep serum was in some way due to antibodies which were haemolytic and agglutinating to mouse erythrocytes and which were present in the normal sheep serum. These antibodies were also present in the immune sheep serum.

Concerning the specificity of various insulins. The general health of the guinea pigs and the sheep which were producing antibodies to insulin was excellent. This observation was consistent with the finding that animals possessing serum of high neutralizing capacity had normal blood-glucose levels. The unimpaired health and normal blood-glucose levels of these animals supports the inference that the antibodies present in the animals did not neutralize endogenous insulin. However, the antibodies did neutralize other insulins injected into the animal. This was shown when twelve immune guinea pigs were found to tolerate an amount of pig insulin which brought about convulsions in all of twelve normal guinea pigs, and when the immune

Table 4. *Effect of repeated injections of immune serum on blood-glucose levels and weights of mice*

On the first day, two intravenous injections of 0.5 ml. and one intraperitoneal injection of 1 ml. were given. On the second and third days, two 1 ml. intraperitoneal injections were given each day. The immune serum used had a neutralizing value of 1 unit/ml.

Day ... Mouse	Blood glucose (mg./100 ml.)			Body weight (g.)		
	2	3	4	1	3	4
Immune serum						
1	248	—	300	18	14	15
2	88	—	267	18	14	14
3	340	—	250	19	16	15
Normal serum						
4	127	—	110	20	17	19
5	95	—	125	16	14	16
6	118	—	70	18	18	18
7	96	—	85	17	15	18
Saline						
8	138	—	120	21	20	22
9	114	—	140	19	20	20
10	167	—	145	22	20	24
11	112	—	105	20	21	21
Immune serum						
12	390	413	—	—	—	—
13	400	438	—	—	—	—
14	180	434	—	—	—	—
15	400	Dead	—	—	—	—
Normal serum						
16	104	82	—	—	—	—
17	92	143	—	—	—	—

Table 5. *Activity of various insulins in the presence of immune guinea pig and sheep sera*

Both guinea pig and sheep sera were prepared using pig insulin as antigen. The criteria for neutralization were prevention of convulsions and effect on blood-glucose levels. Native guinea pig insulin refers to the preparations which were not neutralized by immune guinea pig serum; altered guinea pig insulin refers to the preparation which was neutralized. Neutralized indicated by +, not neutralized by -.

Insulin											
Pig		Ox		Sheep			Guinea pig			Mouse,	Rabbit,
Cryst.	Crude	Cryst.	Crude	Cryst.	Crude	Endogenous	Native	Altered	Endogenous	endogenous	crude
Immune guinea pig serum											
+	+	+	+	+	+	.	-	+	-	+	+
Immune sheep serum											
+	.	+	.	+	+	-	-	+	.	.	+

sheep showed no change in blood glucose level following an injection of a large dose of pig insulin (275 units). When crystalline sheep insulin (20 units) was injected subcutaneously into the immune sheep there was again no alteration in glucose level. This result indicates that the prepared sheep insulin did not revert to endogenous insulin after injection. The summary of the results bearing on the specificity of various insulins given in Table 5 indicates that endogenous guinea pig insulin, and native guinea pig insulin are specifically different from endogenous mouse, altered guinea pig and prepared pig, ox, sheep and rabbit insulin. Endogenous sheep insulin is specifically different from crystalline and crude sheep, crystalline pig and ox, crude rabbit and altered guinea pig insulin.

DISCUSSION

The very fact that pig insulin when used as an antigen can induce neutralizing activity for pig insulin in guinea pig serum justifies the conclusion that insulin and not associated impurities is the effective antigen. This is supported by the fact that insulins of species different from that used as the antigen were also neutralized. Results on anaphylaxis were consistent with the preceding conclusion. However, since crystalline insulin may contain appreciable amounts of associated material (Harfenist & Craig, 1952) it is possible, though highly improbable, that anaphylaxis was not due to insulin.

Guinea pig and the sheep insulins have been prepared which were immunologically different from corresponding endogenous insulins. These altered insulins were neutralized by homologous antisera, whereas the endogenous insulins were not neutralized. Certain preparations of guinea pig insulin were like endogenous guinea pig insulin, in that they were not neutralized by homologous immune serum.

Some consideration was given to the question whether altered insulin could revert to endogenous insulin after injection into the species from which it was derived. It is probable that no such change occurs, since there was no effect on blood-glucose level when altered sheep insulin was injected into an immune sheep. In contrast with this finding, native guinea pig insulin was able to lower blood-glucose levels in spite of the presence of excess guinea pig immune serum.

It is rather striking that guinea pig immune serum, prepared by injecting crystalline pig insulin, neutralized not only a series of prepared insulins including pig, ox, sheep, rabbit and altered guinea pig insulin, but also endogenous mouse insulin. Endogenous mouse insulin, therefore, is immunologically distinct from endogenous guinea pig insulin, and it is probable that endogenous insulins

in general are species specific. Endogenous sheep insulin is specifically different from prepared ox, pig, sheep, rabbit and altered guinea pig insulin. Since native guinea pig insulin was not neutralized by immune sheep serum, no conclusion can be drawn regarding the immunological relationship of native guinea pig and endogenous sheep insulins.

It has been suggested by Lerman (1944) that resistance to insulin in human diabetics is due to specific antibodies and that human insulin would be desirable for the control of such diabetics. However, since the specificity of an insulin preparation can be different from that of its endogenous insulin, for example guinea pig or sheep insulin, it is probable that the effectiveness of a preparation of human insulin for resistant diabetics would depend on whether it was the endogenous type, like native guinea pig insulin, or the altered type.

Some consideration was given to the way in which immune serum rendered mice diabetic. It is most probable that immune guinea pig serum was effective against mouse insulin alone and not against any other component of the mouse, even though other antibodies than anti-insulin may have been developed in the guinea pig in response to injections of crystalline pig insulin. It follows then that diabetes in mice, as indicated by abnormally high blood-glucose levels, loss of weight, and acetone bodies in the urine, resulted from a simple lack of insulin, preceded by no other bodily derangement.

These considerations do not imply that diabetes in humans is caused by an immunity mechanism. It is most unlikely that immunization with endogenous insulin ever takes place. There is of course acquired resistance to insulin in diabetics which in some cases is due to antibodies to heterologous insulin. Fortunately this phenomenon is relatively rare. Smelo (1948) found that a total of fifty-four cases of resistant diabetics had been reported in the English literature. It would appear then, that insulin is a poor antigen in diabetic humans.

Insulin is also a poor antigen in rabbits. Of thirty rabbits repeatedly injected with insulin mixed with various adjuvants, not one produced antibodies demonstrable by the mouse-convulsion technique. On the other hand, little difficulty was experienced in producing immune serum in guinea pigs.

Attempts to demonstrate *in vitro* flocculation in mixtures of insulin and immune serum were unsuccessful.

SUMMARY

1. Intravenous injection of pig or ox or sheep insulin, caused anaphylactic shock in guinea pigs sensitized with crystalline pig or ox insulin.

2. Neutralizing antibodies for insulin were induced in guinea pigs and in sheep by injections

of insulin in Freund's adjuvant. Animals producing antibodies were resistant to insulin. Serum from these animals neutralized insulin, as shown by inoculation into normal mice. This neutralization took place whether the insulin and serum were mixed together before injection or were injected separately at different times.

3. Endogenous guinea pig and sheep insulins were not neutralized by homologous antibodies.

4. Two types of guinea pig insulin have been prepared which were immunologically distinct. One type, classed as altered, was neutralized by immune guinea pig serum. The other, like endogenous guinea pig insulin, was not neutralized. The sheep insulins tested, which included one commercial preparation, were neutralized by immune sheep serum and therefore may be classed as altered.

5. This study indicates that endogenous guinea pig insulin is immunologically distinct from altered guinea pig insulin, from endogenous mouse insulin, and from preparations of pig, ox, sheep and rabbit insulin; and that endogenous sheep insulin is immunologically distinct from altered guinea pig insulin and from preparations of pig, ox, sheep and rabbit insulin.

6. Mice were rendered diabetic by injection of guinea pig anti-insulin serum. These diabetic mice showed abnormally high blood-glucose levels, loss of

weight, and acetone bodies in the urine during the period of administration of serum.

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Arsenolysis and Phosphorolysis of Citrulline in Mammalian Liver

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It has been shown by Knivett (1952*a, b*, 1953*a, b*, 1954*a, b, c*), Slade (1953), Slade, Doughty & Slamp (1954), Oginsky & Gehrig (1952, 1953) and Korzenovsky & Werkman (1953, 1954) that (a) citrulline can react in *Streptococcus faecalis* and other organisms according to the reaction

where ADP and ATP represent adenosine diphosphate and adenosine triphosphate, respectively; (b) citrulline can decompose in the presence of arsenate (by arsenolysis) according to the reaction

phosphate being required in this case; (c) citrulline can be split in the presence of phosphate (by phosphorolysis) according to the reaction

provided that the ornithine formed is removed, e.g. by addition of ornithine decarboxylase.

The present experiments are concerned with analogous reactions in animal tissues. They show that reactions (2) and (3) readily occur in mammalian liver. They are absent from avian liver. The evidence suggests that the reactions are connected with the enzyme system responsible in the intact liver for the synthesis of citrulline from ornithine, CO_2 and ammonia.