

MORPHINE HYPERGLYCEMIA IN DOGS WITH EXPERIMENTAL PANCREATIC DEFICIENCY.

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In a recent investigation¹ we found that after largely abolishing the function of the living pancreas in dogs, without resecting this gland, there was usually no marked hyperglycemia or glycosuria. Occasionally, however, there occurred rises in the glycemia of some of these animals which were greater than those generally observed in the normal dogs. As these animals had less than 5 per cent of un-killed pancreatic tissue remaining and were entirely without any external pancreatic secretion, we looked upon this occasional hyperglycemia as a sign of weakness in the carbohydrate metabolism. The idea then suggested itself that any factor causing a hyperglycemia would probably call forth a greater response in animals with a pancreatic deficiency than in normal individuals. This conception was readily put to an experimental test, and we may state that our expectations were fulfilled.

Method.

Dogs only were employed, and a pancreatic deficiency was produced in four ways. In the first method the pancreas was largely coagulated by injecting alcohol-acetic acid into the main excretory duct, the accessory duct being clamped or ligated. In successful experiments less than 5 per cent of the pancreas remains uncoagulated. In the second group of dogs, the entire pancreas was resected, but the uncinata process with its blood supply intact was transplanted to the subcutaneous tissue in a one stage operation. It should be noted that both classes of dogs were deprived of their pancreatic digestive juices. In another type the pancreas was largely resected, but approximately one-sixth of the gland about the excretory ducts was allowed to remain. Such an animal

¹ Auer, J., and Kleiner, I. S., Preliminary note, *Proc. Soc. Exp. Biol. and Med.*, 1917, xiv, 151.

therefore is not deprived entirely of its pancreatic digestive juices. In the fourth class the uncinata process and tail of the pancreas were merely ligated, leaving about one-third of the structure in connection with the excretory ducts. All operations were performed under full ether anesthesia.

The method for causing hyperglycemia gave some trouble, for we desired a procedure which would not subject the animals to an undue strain, as our first study on the effects of coagulation of the pancreas *in situ* was not completed and we were using the same animals. The method therefore should be as harmless as possible, and in addition should not entail the use of glucose or other sugars in any way. These demands were satisfied by injecting morphine subcutaneously.

The dose of morphine sulfate was small, usually 2 mg. per kilo of body weight, and the site of injection was the subcutaneous tissue of the chest.

All the dogs with an experimental pancreatic deficiency had been operated 1 to 4 months before the morphine test.

As controls we used apparently normal dogs, or dogs which had been fasted for either 8 or 22 days.

All the dogs, except those fasting, were fed once daily a diet composed of about 100 gm. of cooked meat scraps, and 400 to 500 gm. of bread-meat broth mixed with ground bone.

The samples of blood were invariably drawn from an external jugular vein into a syringe containing a small amount of sodium oxalate. In general, four samples of 2.5 cc. of blood were drawn from each dog: a normal sample, and then three more at hourly intervals calculated from the time of the morphine injection.

After obtaining the blood sample, the glycemia was determined by the Lewis-Benedict method as modified by Myers and Bailey.²

RESULTS.

Four dogs, all females, were used in the first series of experiments. Two of them, Dogs AK5 and AK32, were theoretically in the pre-diabetic stage, as at least 95 per cent of the pancreas had been destroyed in each by coagulation 4 months and 1½ months ago respectively. Neither showed a glycosuria. The glycemia in Dog AK5 had ranged between 0.10 and 0.15 per cent; in Dog AK32 between 0.09 and 0.19 per cent, the latter figure being reached only twice before the morphine experiment was made. The two remaining dogs were normal animals and served as controls. All four dogs received 2 mg. of morphine sulfate per kilo subcutaneously in the chest. They had been fed 4 hours before.

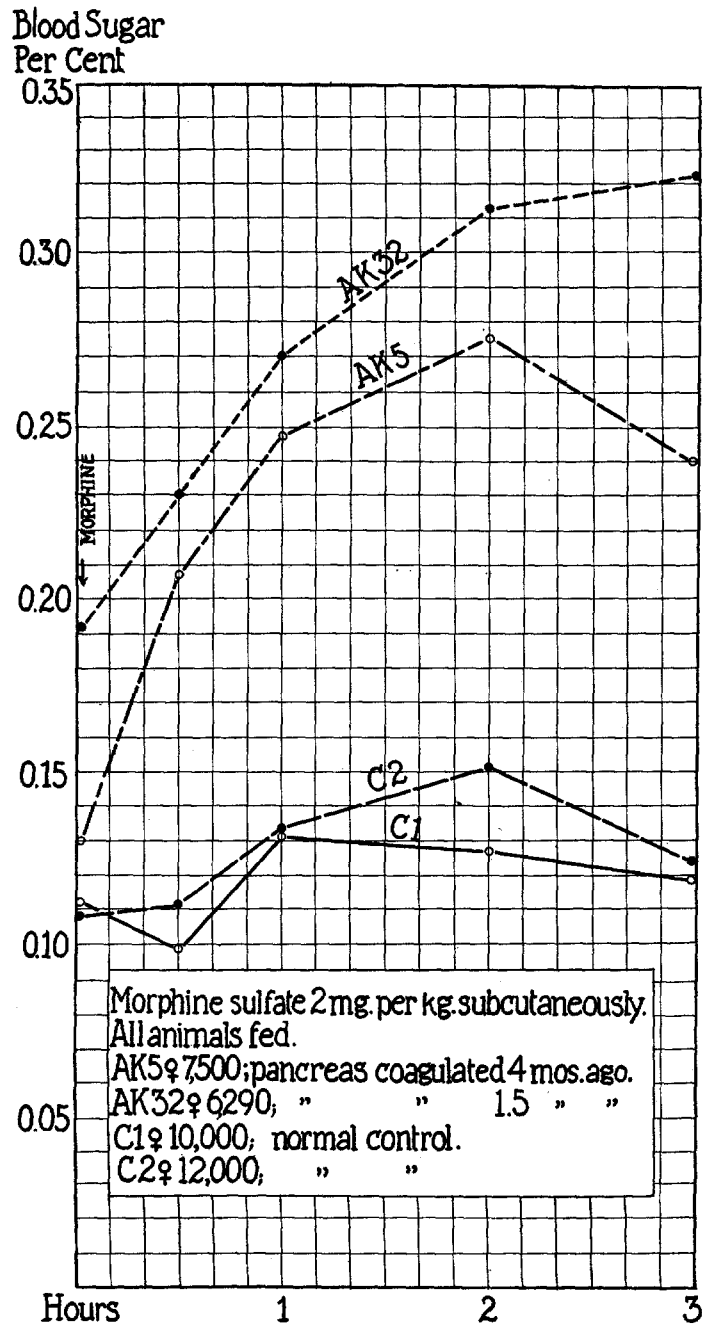
² Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.

The changes in the glycemia are striking and are brought out by the curves of Text-fig. 1. It will be seen that the two controls showed but a slight increase in the glycemia during the 3 hours following the morphine injection, the maximum level reached being 0.15 per cent, an increase of only 0.04 per cent over the normal.

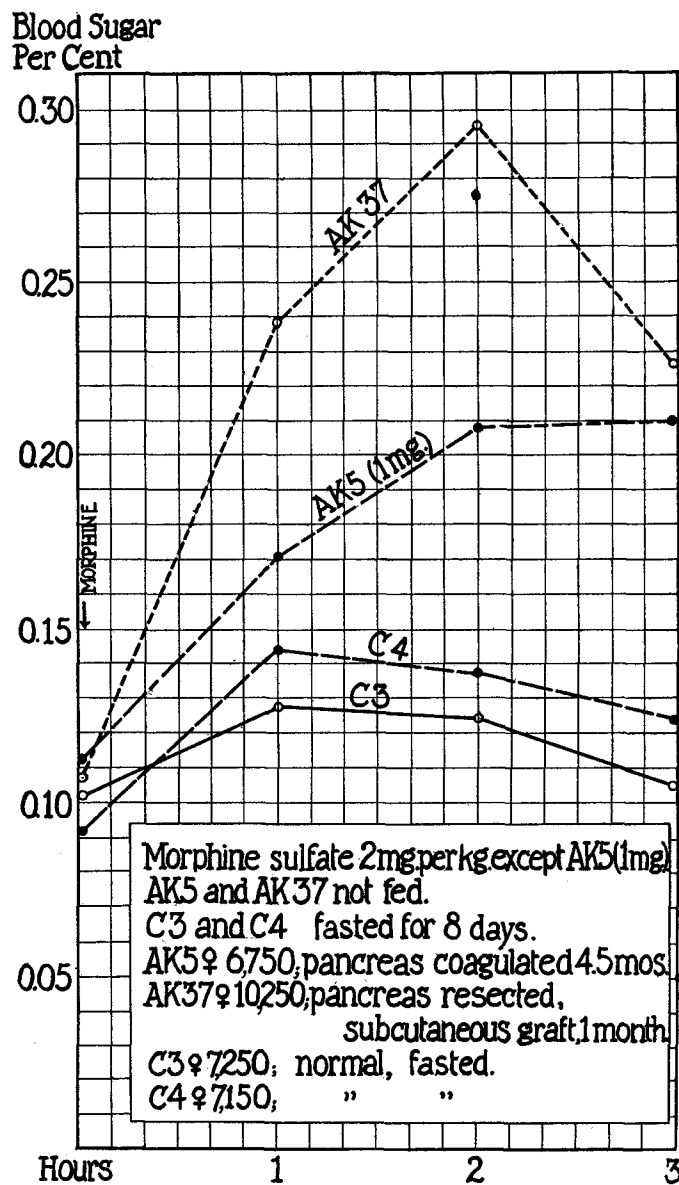
In the dogs with pancreatic deficiency, however, there was a tremendous rise in the blood sugar after the morphine. With Dog AK5 the blood sugar rose from 0.13 to 0.28 per cent after 2 hours; in Dog AK32, from 0.19 to 0.32 per cent in the same length of time. In these animals, therefore, the same dose of morphine caused a rise of 0.15 and 0.13 per cent respectively in the blood sugar, increases which are three to four times greater than those observed in the controls.

In the second series of experiments two dogs with pancreatic deficiency and two controls were employed. One of the prediabetic dogs was again Dog AK5; the second one was Dog AK37 whose pancreas with exception of the uncinata process had been resected 1 month previously, the uncinata portion with intact blood supply being transplanted to the abdominal subcutaneous tissue. The latter dog had no glycosuria beyond an occasional faint trace, and the blood sugar had ranged between 0.09 and 0.15 per cent. The controls were normal animals which had been fasted for 8 days. The prediabetic dogs, Nos. AK5 and AK37, were not fed on the day of the morphine experiment. The two controls and the dog with the subcutaneous pancreatic graft (Dog AK37) received 2 mg. of morphine sulfate per kilo subcutaneously; Dog AK5, however, was only given 1 mg. per kilo.

The results are clearly shown in Text-fig. 2. Here again we observe a striking quantitative difference in the glycemia of the two groups. The control dogs, starting from the 0.09 to 0.10 per cent level, show merely a rise of 0.05 per cent within 2 hours after the morphine. The prediabetic dogs, on the other hand, though beginning practically with the same glycemia as the controls, develop within 2 hours after the morphine a hyperglycemia of 0.21 per cent in Dog AK5 and 0.30 per cent in Dog AK37, levels which represent rises of 0.10 and 0.19 per cent in the blood sugar respectively. It must also be remembered that one prediabetic dog, No. AK5, received only half the amount of morphine per kilo which was given to the controls.



TEXT-FIG. 1. Influence of coagulation of the pancreas on morphine glycemia.



TEXT-FIG. 2. Influence of two types of pancreatic deficiency on morphine glycemia.

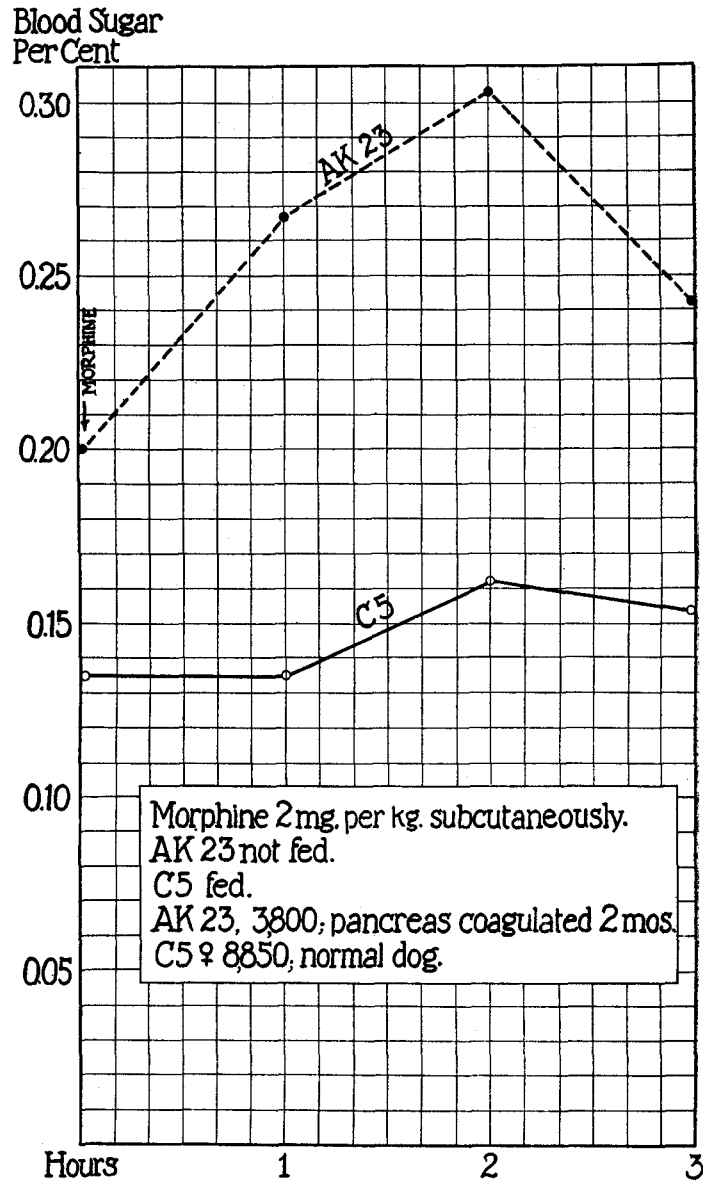
In a third group the effect of 2 mg. of morphine per kilo showed the same general difference described before, and is graphically shown in Text-fig. 3. The pancreatic deficiency in Dog AK23 was caused 2 months previously by coagulating most of the pancreas *in situ* with alcohol-acetic acid. The glycemia of this dog since operation had ranged between 0.120 and 0.20 per cent, the latter figure being reached only occasionally. There was no glycosuria except an occasional faint trace. This dog was not fed on the day of the experiment, but through an oversight the control, Dog C5, was fed 2 hours before. The latter dog vomited a large amount of food within a few minutes after the morphine was given.

From the curves of Text-fig. 3 it will be seen that the control's blood sugar rose from 0.136 to 0.161 per cent within 2 hours after the morphine administration, an increase of 0.025 per cent. The other dog, however, with deficient pancreas showed a glycemia which rose from 0.20 to 0.306 per cent within 2 hours after the morphine, a rise of 0.10 per cent, or four times more than the control.

In a fourth series we studied the effect of morphine when administered to two fasting dogs with the pancreas intact. The fasting period had lasted 22 days, the animals having free access to water. Both dogs weighed originally 8,750 gm. Dog C3 lost 2,900 gm., and Dog C4, 2,750 gm. during the fasting period. The control, Dog C6, was a normal dog weighing 7,500 gm.; it had not been fed on the day of the morphine test. The amount of morphine was 2 mg. per kilo, given subcutaneously as usual.

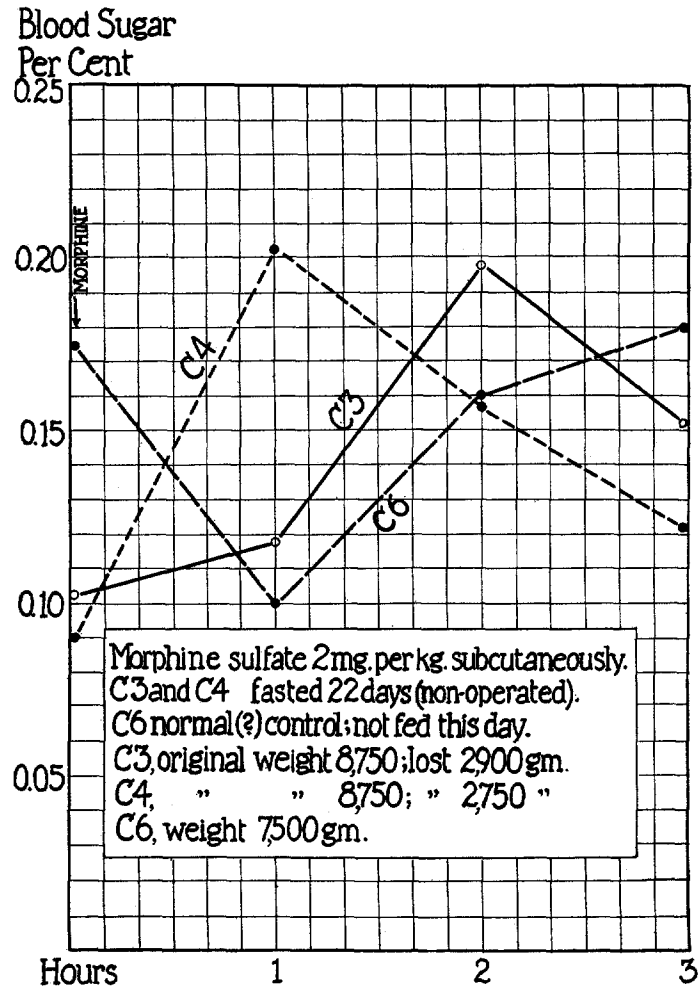
In the fasting animals, Dogs C3 and C4, the morphine produced a considerable rise in the glycemia which was fairly comparable with that observed in the dogs with an experimental pancreatic deficiency. Both dogs, starting at the normal level of 0.090 to 0.104 per cent, showed in 1 to 2 hours after the morphine test, a glycemia of 0.204 and 0.197 per cent, which represent increases of 0.09 to 0.11 per cent. The normal control, Dog C6, showed before the morphine an initially rather high glycemia, 0.175 per cent; 1 hour after the morphine the blood sugar had fallen to 0.10 per cent, but rose again during the next 2 hours to slightly above the original premorphine level. Text-fig. 4 gives these results in graphic form.

The control in this experiment can hardly be considered a normal



TEXT-FIG. 3. Influence of coagulation of the pancreas on morphine glycemia.

animal. The controls in Text-figs. 1 and 2 give a better picture of the response of a normal animal to the subcutaneous injection of morphine.



TEXT-FIG. 4. Influence of prolonged fasting on morphine glycemia in normal dogs.

In a fifth and final series of experiments we employed dogs in which a pancreatic deficiency had been produced by other means than those used in the previous groups. In Dog BD3, a female weighing 7,750

gm., five-sixths of the pancreas had been resected 3 months before, the residual sixth remaining in connection with the excretory ducts, so that the animal had some pancreatic digestion. 3 days after the operation the blood sugar was 0.277 per cent and the urine showed 0.5 per cent sugar; within a few days, however, the blood sugar fell to a normal level, fluctuating between 0.09 and 0.13 per cent, and the urine was sugar-free. In Dog AK40, a female weighing 11,000 gm., the uncinata process and the tail of the pancreas had been ligated off without resection 2 months before. As there was no reason to expect hyperglycemia or glycosuria in this animal, only one blood sugar examination was made a month after the operation; the result was 0.09 per cent. The urine was not examined. Both dogs, Nos. BD3 and AK40, were in excellent physical condition. The control dogs, Nos. C7 and C8, were apparently normal males weighing respectively 6,250 and 7,000 gm. None of the dogs were fed on the day of the morphine experiment. All the dogs of this group received 1 mg. of morphine sulfate per kilo subcutaneously in the chest.

Text-fig. 5 gives the plotted blood sugar curves of this group. The only animal which shows the characteristically prompt, strong rise in glycemia is the dog with partial pancreatectomy, No. BD3. In this dog the blood sugar rose from 0.125 to 0.213 per cent in 50 minutes, an increase of 0.09 per cent.

In Dog AK40, in which portions of the pancreas had been ligated off without resection, the rise of blood sugar did not exceed 0.03 per cent above the premorphine sample.

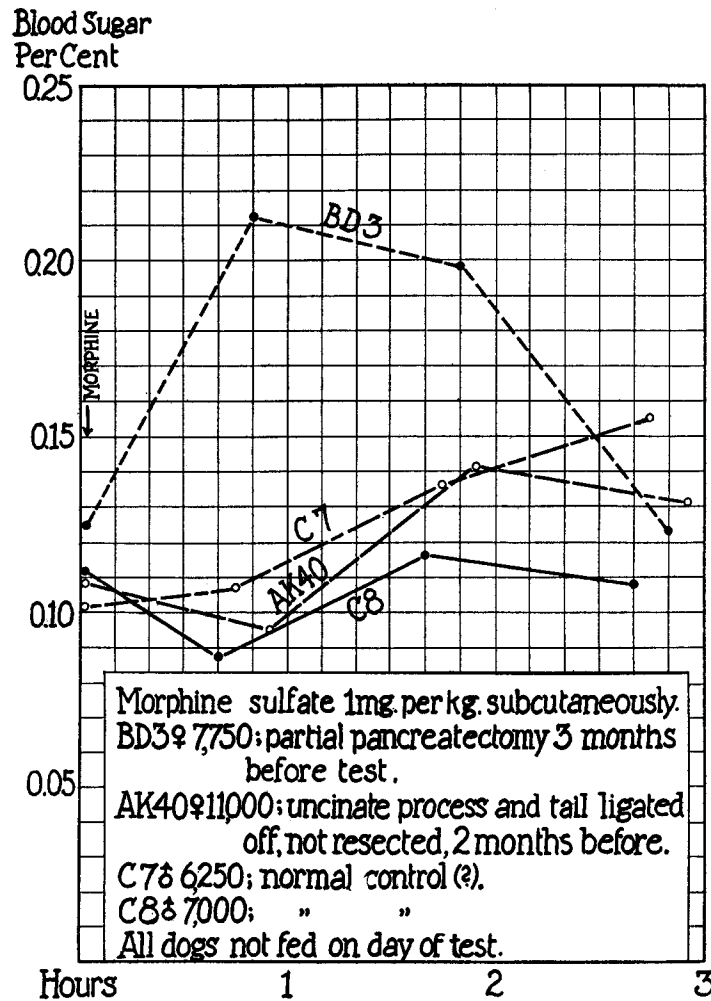
The sugar curve of the controls is quite different from that seen in Dog BD3; in Dog C7 the blood sugar rises slowly after 3 hours to 0.05 per cent above the normal level; in C8 the curve exhibits no rise whatever. The latter animal shows an initial fall of blood sugar after the injection of morphine, but in this instance the blood sample had been taken earlier than in the other dogs. A similar initial fall of the blood sugar after morphine may also be seen in Dog C1 of Text-fig. 1 where a blood sample was taken 30 minutes after the morphine dose.

Urine.—The urine of Dogs AK5, AK23, and AK32 (experiments of Text-figs. 1 and 3) was collected for at least 12 hours after the morphine injection. No sugar was found, except in Dog AK5 (Text-fig. 1) where examination revealed

TABLE I.
Blood Sugar Per Cent before and after Morphine.

| Time. | Text-fig. 1. | | | Text-fig. 2. | | | Text-fig. 3. | | | Text-fig. 4. | | | Text-fig. 5. | | | |
|----------------------|--------------|--------|--------|--------------|---------|--------|--------------|----------|--------|--------------|--------|--------|--------------|---------|--------|--------|
| | Dog AK3 | Dog C1 | Dog C2 | Dog AK37 | Dog AK3 | Dog C3 | Dog C4 | Dog AK23 | Dog C5 | Dog C3 | Dog C4 | Dog C6 | Dog AK40 | Dog BD3 | Dog C7 | Dog C8 |
| Before morphine..... | 0.130 | 0.192 | 0.112 | 0.108 | 0.111 | 0.101 | 0.090 | 0.200 | 0.136 | 0.104 | 0.090 | 0.175 | 0.109 | 0.125 | 0.104 | 0.114 |
| After ½ hr..... | 0.206 | 0.230 | 0.097 | 0.111 | — | — | — | — | — | — | — | — | — | — | — | — |
| “ 1 “ | 0.246 | 0.270 | 0.131 | 0.133 | 0.238 | 0.171 | 0.128 | 0.144 | 0.267 | 0.137 | 0.118 | 0.204 | 0.096 | 0.213 | 0.107 | 0.087 |
| “ 2 hrs..... | 0.275 | 0.315 | 0.128 | 0.151 | 0.297 | 0.209 | 0.124 | 0.138 | 0.306 | 0.161 | 0.197 | 0.157 | 0.141 | 0.197 | 0.136 | 0.115 |
| “ 3 “ | 0.240 | 0.322 | 0.119 | 0.127 | 0.227 | 0.210 | 0.106 | 0.123 | 0.241 | 0.155 | 0.152 | 0.134 | 0.130 | 0.124 | 0.157 | 0.109 |

0.52 per cent sugar in 160 cc. of urine; there was no albumin. The next 24 hour quantity of urine was sugar-free, although 28 gm. of glucose were fed daily beginning with the day after the morphine test. The urine of the other dogs, except



TEXT-FIG. 5. Influence of partial pancreatectomy on morphine glycemia.

Dog BD3 (Text-fig. 5), was not collected. Dog BD3 showed no sugar in the 18 hour urine collected after the morphine test.

Dosage.—The dose of morphine used in these experiments is in all probability larger than necessary. The glycemia curves of Dog AK5 in Text-fig. 2 and of

Dog BD3 in Text-fig. 5 show that a well marked rise in the blood sugar of a dog with pancreatic deficiency may be obtained with only 1 mg. of morphine per kilo. Possibly a dose of morphine can be found which will increase the blood sugar of prediabetic dogs and have no effect on the blood sugar level of normal animals.

How this hyperglycemia after morphine in dogs with a pancreatic deficiency is produced we shall not discuss here. That morphine in larger doses may cause hyperglycemia in dogs is well known.³

General Behavior.—The two groups of dogs of all series exhibited no marked differences in their general response to the morphine injection. All became more or less drowsy; defecation was caused in almost all animals, but retching and vomiting was practically absent in the dogs with pancreatic deficiency and in the fasted controls.

DISCUSSION.

The series of experiments briefly described and figured in the preceding pages show unmistakably that the subcutaneous injection of 1 to 2 mg. of morphine sulfate per kilo of body weight produces a much greater and prompter increase in the blood sugar of dogs with reduced amounts of pancreatic tissue than in normal animals. Text-figs. 1, 2, 3, and 5 and Table I illustrate this well and show the quantitative values obtained in the two classes of dogs.

There is, however, an aspect to this morphine hyperglycemia which may be of practical importance. These dogs with a small or minimal amount of pancreatic tissue may legitimately be considered in a prediabetic stage in the light of much experimental work, especially that of Allen.⁴ On this basis, the morphine glycemia test may be of value to the clinician for detecting patients with a weakened carbohydrate metabolism, thus permitting the early institution of an appropriate dietary in order to prevent the potential diabetes from developing into actuality. The test, moreover, is easily carried out, as less than 1 cc. of blood will be necessary if the Epstein method⁵ is employed. Only three samples of blood, each 0.2 cc. in amount,

³ Hirsch, E., and Reinbach, H., *Z. physiol. Chem.*, 1914, xci, 299–301, Experiments VIII, XI, and XIV.

⁴ Allen, F. M., *Studies concerning glycosuria and diabetes*, Cambridge, 1913.

⁵ Epstein, A. A., *J. Am. Med. Assn.*, 1914, lxiii, 1667. A simplification of the procedure is being developed by one of us which will be ready for publication shortly.

would then be necessary, the normal, control sample, and two further samples taken 1 and 2 hours respectively after the morphine injection. The amount of morphine given to the human subject cannot, of course, be calculated kilo for kilo from the doses used for dogs; probably 20 mg. of morphine sulfate ($\frac{1}{3}$ grain) would suffice for an adult.

Whether the morphine test will yield the same result with human beings in the prediabetic stage which we obtained experimentally in dogs, only actual trial can determine. Such a trial, however, we believe warranted by our results and by the simplicity of the procedure. The injection of a moderate dose of morphine is surely not more of a strain to the organism with a possibly defective carbohydrate metabolism than the ingestion of 100 to 200 gm. of glucose; moreover, it will be remembered that morphine has been and is administered to diabetics with apparently beneficial results. Thus, for example, Pavy⁶ reported that opium, morphine, and especially codeine reduce the glycosuria in human diabetes. It is therefore unlikely that morphine will work harm in the prediabetic stage of human diabetes.

When to suspect the prediabetic stage in a patient will offer no difficulties to the physician. The combination of racial or family predisposition, neurotic temperament, rheumatoid pains, and periods of muscular weakness point to a possibly defective carbohydrate metabolism, though not associated with a glycosuria. If there is furunculosis, or pruritus, or increased thirst and micturition, or early development of impotence, a prediabetic stage is to be suspected, even though the urine is sugar-free. In such cases the morphine test is worthy of a trial.

Sugar Tolerance.—It should be mentioned that the sugar tolerance of the dogs in which the pancreas had been coagulated by alcohol was surprisingly good. For example, Dog AK5, 90 days after operation excreted only 0.3 gm. of sugar per kilo after being fed 10 gm. per kilo. From the 119th day to the 175th day the same dog was fed daily, except on the day of the morphine test, 4 gm. of sugar per kilo,

⁶ Pavy, F. W., *Guy's Hosp. Rep.*, 1870, xv, series 3, 420. It may be noted that as much as $2\frac{1}{4}$ grains (165 mg.) of morphine hydrochloride were administered three times a day to Pavy's Case 3 (p. 430).

in addition to the regular mixed diet, but no sugar appeared in the 24 hour urines.

Another example is furnished by Dog AK32. This dog showed a severe diabetes during the 1st week after the operation: the glycosuria varied between 2.7 to 4.8 per cent and the blood sugar ranged from 0.16 to 0.32 per cent. Within 2 weeks the urine became sugar-free and the glycemia oscillated between 0.09 and 0.17 per cent. A tolerance test on the 21st day after operation (10 gm. of sugar per kilo *per os*) caused no sugar excretion whatever. Thereafter this animal's urine up to the time of death, 118 days after operation, never showed any sugar, beyond an occasional faint trace. It is therefore evident that a strong hyperglycemia after small doses of morphine can even then be obtained when the carbohydrate metabolism is only moderately impaired.

Fasting.—In dogs which had fasted sufficiently long, the subcutaneous injection of 2 mg. of morphine sulfate per kilo sufficed apparently to bring on a definite hyperglycemia. This is shown in Text-fig. 4. In the two animals, Dogs C3 and C4, which had fasted 22 days, the subcutaneous injection of 2 mg. of morphine sulfate per kilo produced in 1 to 2 hours a hyperglycemia of 0.20 per cent, the normal level of the same animals being 0.09 to 0.10 per cent. Since the dogs with deficient pancreas, excepting Dog BD3 (Text-fig. 5), were losing weight constantly in spite of a liberal mixed diet, because they were devoid of pancreatic digestion, it might be objected that the morphine hyperglycemia which we have described merely indicates a fasting state and not a weakness of the carbohydrate metabolism, as we have assumed. This interpretation, however, is probably only partially correct at best and by no means decreases the value of our results. It has been well known since the time of Claude Bernard that fasting or cachectic animals in general may respond with a transitory glycosuria to the ingestion of a full meal of carbohydrates, but it is clear that such an alimentary glycosuria must be due to a temporarily weakened carbohydrate metabolism, for the normal organism would show a sugar-free urine. Therefore the morphine hyperglycemia which we observed during fasting is additional evidence for the correctness of our working hypothesis that morphine

will cause a greater hyperglycemia in an animal with impaired carbohydrate metabolism than in a normal individual.

On the whole, therefore, it may be said that the morphine hyperglycemia during severe fasting is not only no evidence against the correctness of our hypothesis but is, on the contrary, just what that view demands. Furthermore, it must be emphasized that a pancreatic deficiency without obvious fasting, as in Dog BD3 (Text-fig. 5), also causes a well marked hyperglycemia when a small dose of morphine is injected subcutaneously; severe fasting *per se*, therefore, is also not a necessary factor for the appearance of a marked hyperglycemia after morphine in prediabetic dogs.

It should be observed that a moderate degree of fasting is insufficient to bring out the morphine hyperglycemia. Thus a fasting period of 8 days in Dogs C3 and C4 did not cause a marked hyperglycemia after morphine, as the curves of Text-fig. 2 show.

SUMMARY.

The subcutaneous injection of 1 or 2 mg. of morphine sulfate per kilo subcutaneously in dogs with a pancreatic deficiency, whose sugar tolerance is still good, produces a rise in the glycemia about four times greater than the same amount of morphine calls forth in normal dogs.

As dogs with a pancreatic deficiency due to coagulation or partial resection of the gland may legitimately be considered in a prediabetic state, the inference is warranted that the morphine test may be of value in detecting a weakened carbohydrate metabolism in the human subject. The test could easily and without danger be carried out with the micro methods now available for the quantitative determination of blood sugar.

The experimental facts described in this paper give additional corroboration to the view that the response of a normal and of a pathologically altered organism to the same drug in the same dosage may be quantitatively very different.