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THE EFFECT OF ANESTHESIA (ETHER, AMYTAL, PENTOBARBITAL), OF FASTING, AND OF SPLENECTOMY UPON THE BLOOD CHEMISTRIES OF THE RAT: HEMOGLOBIN, NPN, GLUCOSE, ALBUMIN, GLOBULIN AND CHLORIDE.

By

HENRY I. KOHN, NACY SWINGLEY, AND MARY NELL CUPP

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Biology Division

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ABSTRACT

THE EFFECT OF ANESTHESIA (ETHER, AMYTAL, PENTOBARBITAL), OF FASTING, AND OF SPENECTOMY UPON THE BLOOD CHEMISTRIES OF THE RAT: HEMOGLOBIN, NPN, GLUCOSE, ALBUMIN, GLOBULIN AND CHLORIDE

Henry I. Kohn, Nancy Swingley, and Mary Nell Cupp

Determinations of hemoglobin, and of plasma NPN, albumin, globulin, glucose, and chloride were made in Sprague-Dawley adult male rats under various conditions. The blood was obtained by cardiac puncture.

Amytal acts more quickly and briefly than pentobarbital. These drugs cause a transient fall in hemoglobin, total protein and glucose. With pentobarbital, the fall in glucose outlasts the fall in hemoglobin and protein. As the animals recover from the anesthesia, the values of all the determinations gradually equal those obtained under brief (less than two minutes) ether anesthesia.

Splenectomy slightly lowers the chloride, and perhaps some of the other levels. It abolishes the fall in total protein under amytal anesthesia. It does not prevent the transient fall in hemoglobin or glucose, but somewhat slows recovery.

Withholding food, but not water, causes a parallel decrease in the total protein, NPN, and glucose, which is complete in about two days. Thereafter the levels remain constant for five days. During this later period, however, the A/G ratio rises slightly. Animals that have been starved for 1 - 3 days require about twice as many days of refeeding to recover the normal levels, and about three times as many to achieve the control weight.

THE EFFECT OF ANESTHESIA (ETHER, AMYTAL, PENTOBARBITAL), OF FASTING, AND OF SPLENECTOMY UPON THE BLOOD CHEMISTRIES OF THE RAT: HEMOGLOBIN, NPN, GLUCOSE, ALBUMIN, GLOBULIN AND CHLORIDE.

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In the course of our studies upon the metabolic response of the rat to whole body X-irradiation, it was necessary to choose a standardized set of conditions for the control of blood sampling. Reference to the literature, which for the rat is very scant, revealed several points.

Since the work of Page (1), amytal and other barbiturates have been employed for anesthesia when blood sugar or liver glycogen is to be determined. These agents tend to prevent mobilization of carbohydrate, as Evans, Tsai and Young (2) showed, by blocking or preventing stimulation of the splanchnics.

Essex et al (3) concluded that in dogs under amytal the spleen extracts erythrocytes from the circulation and swells, whereas under ether it contracts. Bonnycastle (4) showed that in dogs under pentobarbital the plasma volume rises, while the blood volume remains constant. Carr and Essex (5) provided quantitative data on the effect of splenectomy. In dogs which had received 25 - 30 mgm. per kilo i.v. of Na pentobarbital, the hemoglobin fell some 20 per cent and remained low for about 3 hours. In the splenectomized dog, this dosage produced a 7 per cent drop for one hour. Wakim and Mason (6) found pentobarbital to lower both hemoglobin and plasma protein in the dog. In the rat, according to Higgins and Corwin (7), amytal compared to other produced a fall in both red and the white cell counts. Splenectomy was without effect upon the red cell count, though it tended to eliminate the fall in the white. In the rabbit, amytal caused a fall in the white cell count only, and this was not affected by splenectomy. More recently, Quimby and Saxon (8) observed a falling red cell count and plasma specific gravity under both amytal and ether in the rat.

As for changes in the rat during starvation, the fall of glucose and the rise of ketone bodies are well known. Cutting and Cutter (9) followed the proteins for two weeks during starvation. The plasma volume gradually fell about 30 per cent, and after the first week, the concentration of serum proteins fell about 15 per cent. We have found no detailed studies on the electrolytes.

The foregoing are sufficient to warrant that different values of the blood chemistries will be obtained when a variety of conditions for blood sampling are employed, but are not sufficient to predict what these might be. It was therefore decided to check each determination in the normal and splenectomized animal, fed or starved, under ether, amytal or pentobarbital.

METHODS

The animals employed were Sprague-Dawley male rats, which in most of the experiments weighed around 250 - 300 grams. Blood was obtained by cardiac puncture, using a half-inch No. 23 needle and a syringe which had been rinsed with 1 per cent heparin in saline. The animals were always bled between 8:30 and 10:30 a.m. About 2.5 ml. of blood were placed in a small centrifuge tube in which 0.1 mgm. of heparin had been dried, and after taking .02 ml. for the hemoglobin determination, the rest was centrifuged and the plasma employed for the other determinations. All methods used the Coleman junior spectrophotometer.

Hemoglobin was determined as oxyhemoglobin by adding .02 ml. of whole blood to 5 ml. of 0.1 per cent Na_2CO_3 and reading at 545 mu. The factor of 46.2 was determined by testing samples of known hemoglobin content. The latter were standardized by the alkaline hematin method using a commercial hematin standard prepared according to Horecker (10). This, in turn, was compared with the result of several direct iron determinations on blood according to Wong (11), which gave a value 4 per cent higher.

The tungstic acid filtrate of plasma was made by mixing 0.5 ml. of plasma, 3.0 ml. of water and 2.0 ml. of the tungstic acid reagent. The latter was prepared freshly each day by adding 2.5 parts of 10 per cent Na tungstate to 17.3 parts of 0.147 n sulfuric acid. This adjusted the pH of the filtrate to 2.5, which we found optimal for protein precipitation as judged by obtaining a minimal value of the NPN in the Kjeldahl. The Kjeldahl was done by the sulphuric acid-hydrogen peroxide method, and nesslerized.

Glucose was determined by the arsenomolybdate method of Nelson (12) read at 520 mu.

Chloride was determined by the Van Slyke and Hiller (13) modification of the Sendroy method. We employed the colorimetric modification suggested by the Aloe Technical Manual in which the 2 ml. aliquot, titrated by Van Slyke and Hiller, is made up to 50 ml. with distilled water plus 200 mgm. of sodium iodide and read in the photometer at 500 mu.

Proteins were determined by Weichselbaum's (14) modification of the biuret test. The method was standardized against the Kjeldahl. For the precipitation of globulin and fibrinogen we employed sodium sulfite, as suggested by Campbell and Hanna (15), who found the reagent to act independently of temperature. To be sure that this would apply to the rat, we carried out precipitations at 22° and 37° C. and covered the range of concentrations from 10 to 28 per cent of sodium sulfite. The curve relating precipitation to final salt concentration showed a discontinuity in the form of a small plateau between 19 and 22 per cent sodium sulfite. We therefore selected 19.5 per cent as our standard working concentration. The curves obtained at 22° and 37° were identical.

It may be pointed out that the titers we have found differ from those frequently seen because they are for plasma, and not whole blood, obtained from the heart.

EXPERIMENTS

Experiments on Anesthesia

We examined the effects of ether, Na amytal and Na pentobarbital (nembutal) because they seemed to be the most convenient. For ether, a 2-liter glass jar with an adequate cover was employed, on the floor of which was a large cotton wad containing ether. Rats weighing about 200 grams were in the third stage of anesthesia in less than 2 minutes after entering the jar. They were removed and cardiac puncture was effected in the course of another one or two minutes, without further anesthesia, before recovery. Occasionally it was necessary to prolong anesthesia. This was done by placing the animal's nose at the open end of a 100 ml. beaker, the bottom of which was packed with cotton containing ether. In our experience, ether is the simplest anesthetic for the purpose at hand, and also involves the most rapid recovery.

Normal values for the various blood tests obtained under ether anesthesia are listed in Table I. The animals of this table served as controls at various times during the course of the present experiment, and varied in weight from 200 to 400 grams. The data are very consistent, perhaps surprisingly so in some cases. It may be remarked that all of the animals had been in the animal farm for some time before use and that blood was drawn only between 8:30 and 10 a.m. It should also be noted that each datum is the average for a pair of animals. In the Long-Evans strain, using serum, Leathem (16) found a total protein of 6.34 per cent, an A/G ratio of 1.56, and an NPN of 49.8 mgm. per cent.

Sodium amytal was chosen because it is soluble and is a relatively short acting barbiturate. We have given it intraperitioneally, as a 5 or 6.5 per cent aqueous solution, which we found stable for at least 5 days when kept in the refrigerator. However, the experiments reported here involved freshly prepared solutions only. In our experience, deep anesthesia suitable for abdominal

Table I

Blood Levels Under Ether Anesthesia

The tests were performed on cardiac blood (hemoglobin) or cardiac plasma (total protein, albumin, glucose, NPN, and chloride) obtained from unstarved adult Sprague-Dawley rats. Each datum is the average for the analyses of two rats.

Item	Hemoglobin	Total Protein	Albumin	A/G	Glucose	NPN	Chloride*
	gm.%	gm.%	gm.%		mgm.%	mgm.%	mgm.%
Average	17.8	7.04	4.72	2.03	169	42.4	609
Standard deviation	0.1	.08	.05	.01	1.7	1.5	4.0
No. of pairs	12	12	6	6	12	7	7

* Chloride as NaCl.

surgery is not obtained conveniently with this drug, though simple short operations such as splenectomies can be performed with it. The full anesthestic dose lies in the range 80 - 110 mgm. per kilo.

After exploring the range from 50 - 70 mgm. per kilo, we chose 65 mgm. as providing satisfactory light anesthesia and rapid induction for cardiac puncture in both sexes and in all rats weighing more than 125 grams (the younger ages were not tested). At this dosage, anesthesia begins in about 5 minutes and is over in less than 25. Na pentobarbital was taken for comparison because it offers the possibility of heavier and longer barbiturate anesthesia; we used 50 mgm. per kilo because it is quite safe for both sexes and a variety of ages. Higher dosage is not quite safe, while lower ones act less completely. Anesthesia begins at about 10 minutes and is over in less than 40. Females show a tendency to be more sensitive to both drugs.

For our purposes, the gross effects of anesthesia may be divided into four stages: Stage 1, the animal moves about with faulty vestibular function. Stage 2, the animal lies quietly, but if stimulated will move about. Stage 3, the animal appears unconscious, but responds reflexly to pain (e.g., pinching the tail). Stage 4, deep anesthesia.

With 65 mgm. per kilo of amytal, i.p., stage 3 is reached in 5 - 8 minutes; stage 2 is entered at 25 - 30 minutes; stage 1 is entered at 30 - 40 minutes. Under 50 mgm. per kilo of pentobarbital, i.p., stage 4 is reached in 5 - 10 minutes; stage 3 begins at 45 - 60 minutes, and stage 1 is reached at about 90 minutes.

The chemical data are summarized in Figures 1 and 2, which show striking changes in glucose, total protein, and hemoglobin. Each of these fell during the early period of barbiturate anesthesia, and subsequently rose to equal the value obtained under ether. The value for ether is that of the unanesthetized excited rat. In the mouse, Hiestand et al (17) found chloroform to give the same blood sugar value as the unanesthetized control (174 mgm. per cent). In

Figure 1

Cardiac plasma glucose as a function of time after administration os anesthestic. The values for ether at 1.5 min. are given in the upper left hand corner. Note that pentobarbital takes about 30 minutes to reach the low value attained by amytal in 10 min. As anesthesia lessens, the titer approaches and finally reaches that obtained under ether. The data were obtained on five separate days, as indicated by the shape of the symbols. Each point is the average of two different animals.

FIG. 1

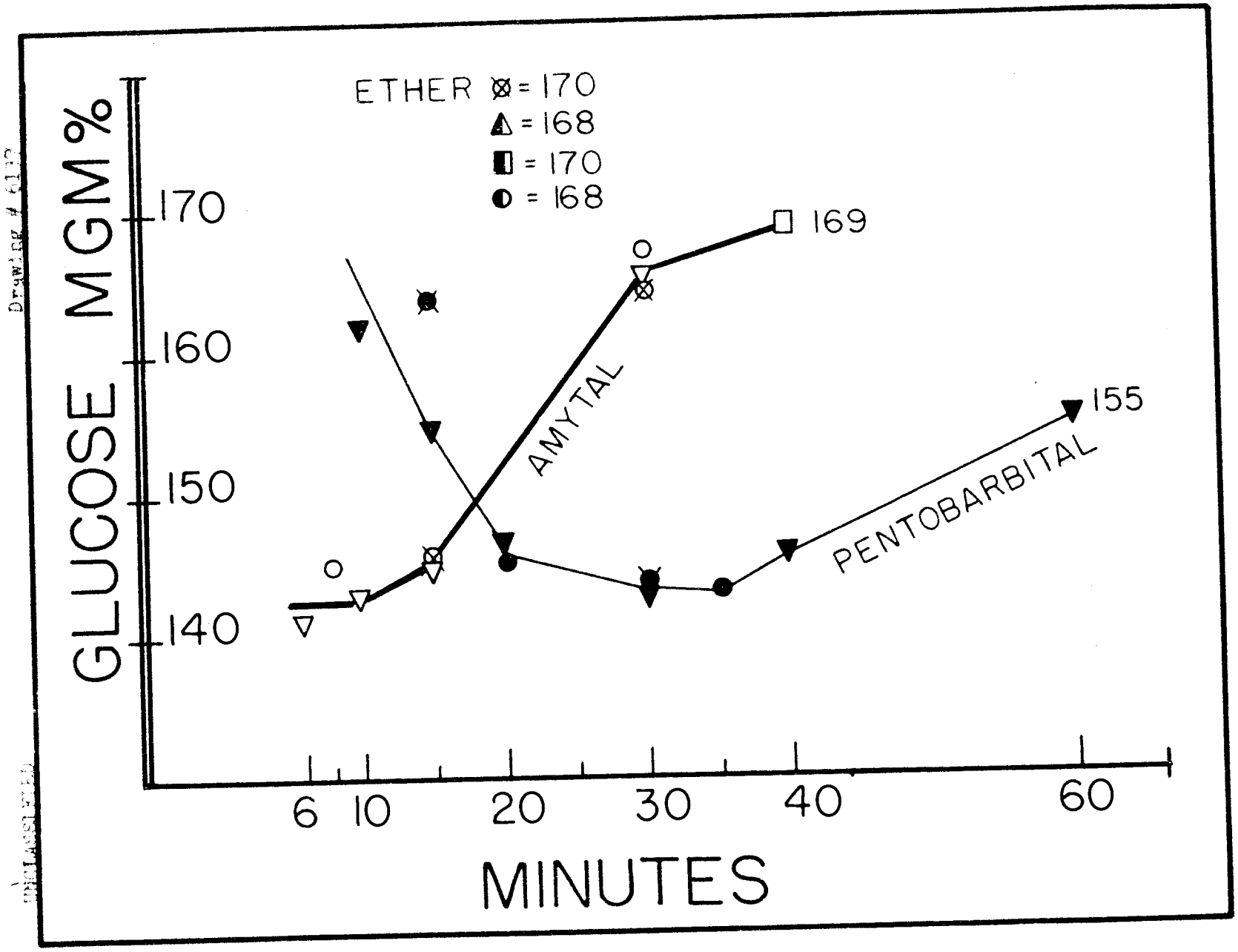
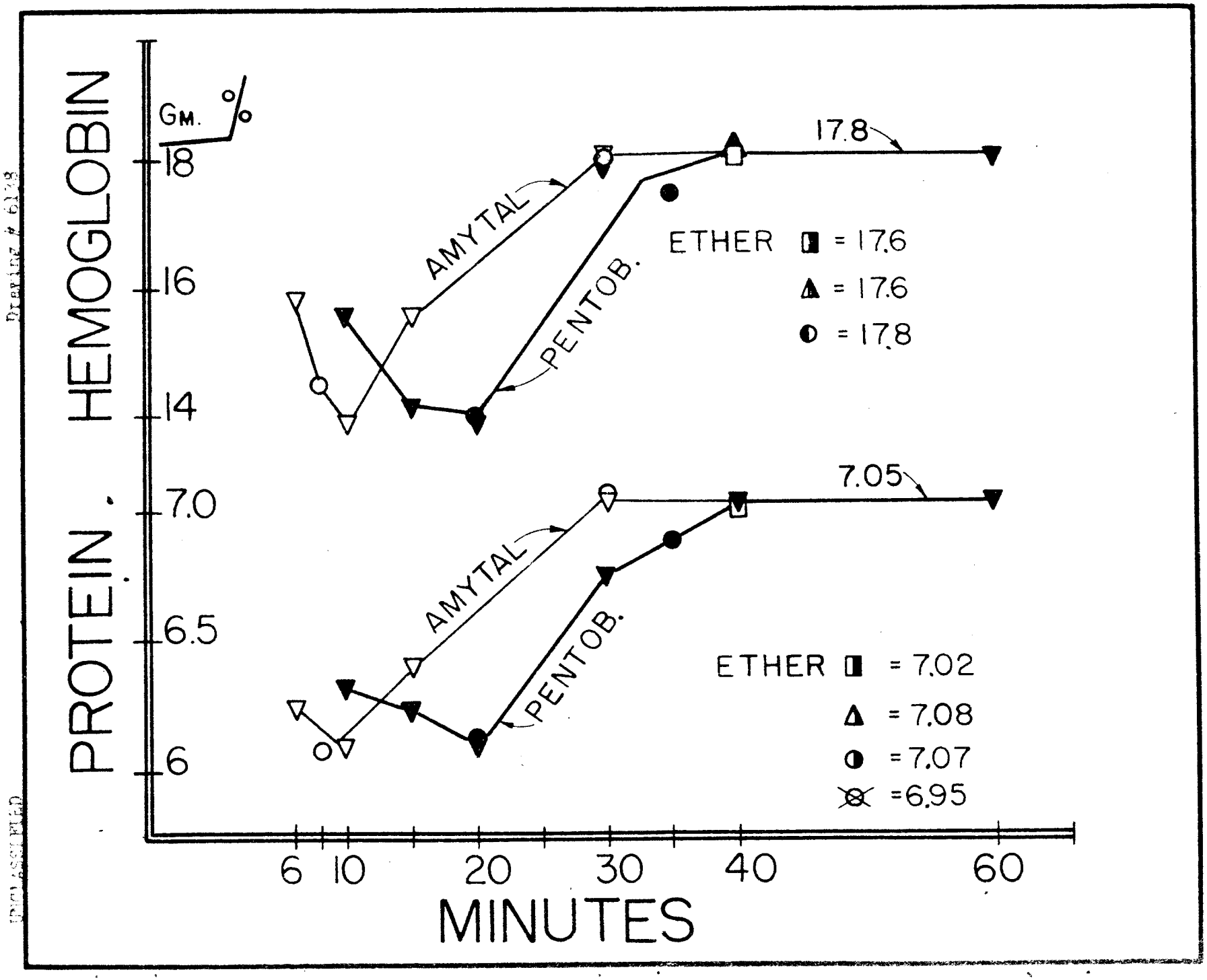

ETHER ⊗ = 170
▲ = 168
◨ = 170
◐ = 168
GLUCOSE MGM%
170
160
150
140
169
155
AMYTAL
PENTOBARBITAL
6
10
20
30
40
60
MINUTES

Figure 2

Cardiac plasma protein and blood hemoglobin as a function of time after administration of anesthetic. The values for ether at 1.5 minutes are given in the lower right hand corners. The values of both hemoglobin and protein run parallel courses under the two anesthetics, falling at first, then rising to a final steady value equal to that obtained under ether. The changes under pentobarbital occur more slowly. The A/G ratio was determined in every case, and was found to be constant (1.98 - 2.06). Each point is the average of two different animals. The data was obtained on five different days, as indicated by the shape of the symbols.

FIG. 2


PROTEIN . HEMOGLOBIN
GM. %
18
16
14
7.0
6.5
6
AMYTAL
PENTOB.
17.8
ETHER = 17.6
= 17.6
= 17.8
7.05
ETHER = 7.02
= 7.08
= 7.07
= 6.95
6
10
20
30
40
60
MINUTES

man, Epstein and Aschner (18) found approximately a 50 per cent rise in blood sugar during major surgical procedures under ether-nitrous oxide.

Glucose acted independently of total protein and hemoglobin. Under amytal it reached a low of 140 - 145 mgm. per cent 5 - 10 minutes after drug administration, and had returned to the ether level of 165 - 170 mgm. per cent at 30 minutes. Under pentobarbital, however, the low value was not reached until the interval 20 - 40 minutes, and the "ether value" was not yet obtained at 60 minutes, although the animals were in stage 2 of anesthesia (as defined above).

Total protein and hemoglobin underwent parallel changes. Under amytal, these more or less paralleled the plasma glucose, reaching a low at about 10 minutes, and attaining the ether level by 25. Under pentobarbital, 15 - 20 minutes were required to reach the minimum, and 35 minutes to reach the ether level, at which time, however, the glucose was at its lowest. The A/G ratio was constant throughout (1.98 - 2.06).

The percentage change, comparing the lowest to the final highest value, was about 20 per cent for hemoglobin and 15 per cent for plasma protein. The plasma NPN also showed a tendency to fall and then to rise, but the change was less than 8 per cent. In one experiment, the ether value was 44.4 mgm. per cent; amytal at 8 minutes was 42, and at 30 minutes 43.4; pentobarbital at 20 minutes was 41, and at 35 minutes 43.2. On the other hand, the values for plasma chloride were constant to within 2 per cent.

It thus appears that the cardiac plasma values for the rat coming out of amytal or pentobarbital anesthesia are identical with those under full ether anesthesia, and presumably are equal to those for the excited unanesthetized rat. Low values of sugar, of protein and hemoglobin, or of both can be obtained by varying the time and the anesthetic.

Experiments on Splenectomized Animals.

The data of the previous section, establishing a transient fall in the hemoglobin, plasma protein and NPN, suggest a shift of fluid from one

compartment to another under the influence of the barbiturates. On the other hand, the spleen was implicated in the dog, though Higgins and Corwin (7) found its removal to be without effect upon the red cell response to amytal in the rat.

To check on the role of the spleen, we splenectomized about 20 Sprague-Dawley males, weighing between 350 ± 45 grams, and after 2 - 3 weeks, when all had surpassed their preoperative weights, examined their blood during the course of ether and amytal anesthesia. After the experiment, the animals were sacrificed, and it was established that neither inflammation nor an appreciable amount of splenic tissue was present in any of the operated animals.

The data from two separate experiments were utilized to construct Table II. Each datum is the average of two animals. None were used twice. The data of the controls were consistent with those previously obtained except in one respect: the fall in hemoglobin under amytal is smaller. This might be explained by the fact that the present series was considerably older (average weight of 350 compared to 250 grams).

The effect of splenectomy itself must be considered first. According to the top part of Table II, hemoglobin, total protein, chloride and NPN were decreased slightly. There was no change in the A/G ratio or glucose. The lower part of the table deals with the reaction to amytal. The A/G ratio and NPN were unaffected. The chloride data were also unchanged and were very consistent, so that the small fall due to splenectomy appears quite significant.

The fall of hemoglobin under amytal is actually greater in the splenectomized animals than in the controls of this series, though not so great as in the previous younger series. This confirms the work of Higgins and Corwin (7) and establishes the reaction of the rat as different from the dog. Recovery to the normal level was delayed.

The greatest change induced by splenectomy was in the total protein, which failed to react under amytal anesthesia.

Table II

The Effect of Splenectomy

Two separate experiments are combined in the table. Each datum is the average of two animals, none of which was used twice. The determinations were on cardiac plasma.

Anesthetic	Type	Time	Hemoglobin	Total Protein	A/G	Chloride	Glucose	NPN
		min.	gm.%	gm.%		mgm.%	mgm.%	mgm.%
Ether	Control	-	18.3	7.04	2.01	605	168	41.8
Ether	Splenectomized	-	17.6	6.72	2.01	583	168	39.3
			17.8	6.67	1.96	581	167	40.4
Amytal	Control	8	17.2	6.18	2.01	604	147	40.4
			17.6	6.40	1.99	605	143	40.4
		30	18.3	7.10	2.01	604	167	41.5
Amytal	Splenectomized	8	15.6	6.8	2.02	583	143	39.3
			16.7	6.54	2.01	581	147	39.3
		15	16.7	6.73	2.01	583	149	39.3
		30	16.7	6.54	2.01	586	154	39.5
			16.9	6.8	2.02	589	159	40.4

The initial fall in glucose was not prevented by splenectomy, but recovery was delayed.

In summary, then, splenectomy in the rat lowers the level of certain blood constitutents and changes the response to amytal anesthesia. Unlike the situation in the dog, hemoglobin still falls, though its subsequent rise, and also that of glucose, is delayed. This change may be due to the altered circulation of the liver which, following splenectomy, loses the flow from the splenic vein. The most striking difference in the operated animal is the failure of the plasma proteins to change under amytal. This may be due to the altered hepatic circulation, or perhaps to the loss of a splenic function.

Experiments on Fasting.

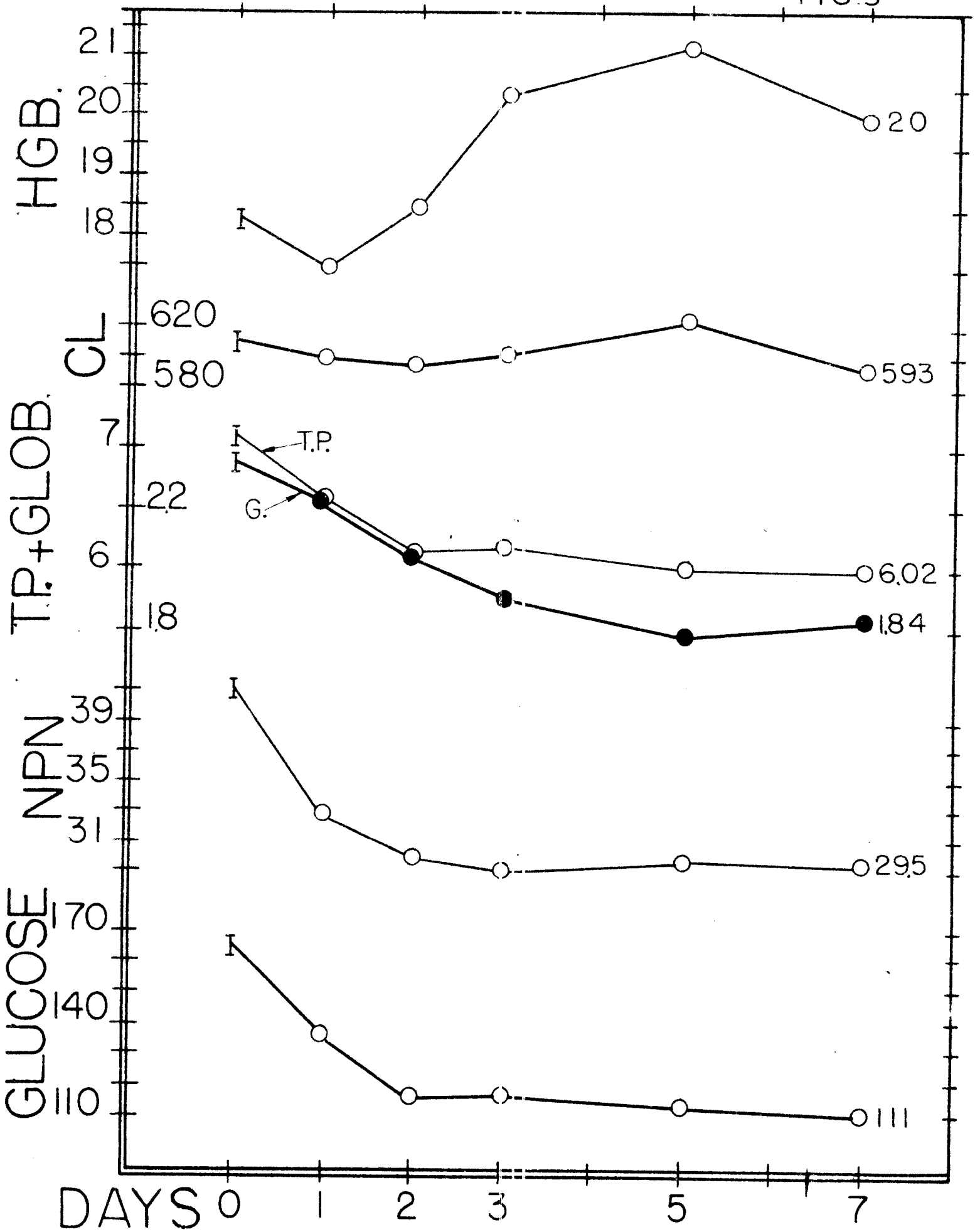
The reaction of the rat to fasting was followed in two experiments. The first involved 28 males averaging 250 grams, of which 5 were used as controls. Food was withdrawn (but not water) on Day 0, and groups of 3 were examined at 24 hours (ether), 72 hours (ether and amytal), and 120 hours (ether and amytal). The data showed that the plasma protein, glucose and NPN fell during the course of several days, that the A/G ratio rose somewhat after several days, and that amytal lowered both protein and glucose in the starved animal. Eight animals which had been starved were given food again for varying periods before analysis, and will be discussed later.

The second experiment was designed in accordance with the findings of the first, and confirmed it. The animals weighed between 300 and 350 grams. In order to minimize the relative errors of analysis, all of the analyses were done for the starvation series on the same day, food having been withdrawn from the individual groups 7, 5, 3, 2, 1, and 0 days previously. The data are summarized in Figure 3, where each point represents the average of one pair of animals. The barred points at Day 0 represent the maximum spread of the averages of 3 pairs, one analyzed on the same day as the entire starvation series, the

Figure 3

Hemoglobin and cardiac plasma chemistries as a function of time after withdrawal of food, but not of water. The points at Day 0 are based on the average of 3 pairs of animals; on each of the other days a single pair was analyzed.

FIG. 3


HGB.
21
20
19
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20
CL
620
580
593
T.P.+GLOB.
7
6
22
18
T.P.
G.
6.02
1.84
NPN
39
35
31
29.5
GLUCOSE
170
140
110
111
DAYS
0
1
2
3
5
7

other two being the control analyses performed 2 and 5 days later during the course of the refeeding experiment. It is obvious that the differences are highly significant.

The data show at most a small change in chloride, which need not be considered. The other items showed significant changes, which in the case of the proteins, NPN and glucose paralleled one another. These required about 2 days or a little more to become constant. The total protein fell about 1 gram during the first 2 days, after which the A/G ratio rose from 2 to about 2.25. The plasma NPN fell from about 41 to 29 mgm. per cent during the first two days, and was constant for the rest of the week. The glucose fell from about 170 to 116 mgm. per cent in 2 days, and by the end of the week was at 111. While the change in the NPN and glucose must have been partly accounted for by the decreased absorption of material from the G. I. tract, the latter could hardly account for the requirement of 48 hours to stabilize the plasma levels. Remarkable, too, is the fact that the plasma proteins showed parallel changes during this period. Such data, it would seem, represent the change in activity of the homeostatic mechanisms, presumably under endocrine control, which set the level of the various blood constituents.

It is of interest to compare the blood sugar of the mouse during fasting with the present results. Marshak et al (19), using tail blood from unanesthetized animals, found a "fed" value of 138 mgm. per cent, which during fasting fell to 73 after two days, 81 after three days, and 82 after four. Dwarf mice, lacking the pituitary acidophils, had a "fed" value of 120 mgm. per cent, which fell to 69, 82, and 84, respectively, after 2, 3, and 4 days of fasting.

The changes in the hemoglobin concentration did not parallel the others. Starting at about 18.3 grams per cent, it fell about 4 per cent during the first day, returned to normal on the second, and thereafter remained between 8 and 13 per cent above the original. Presumably this represents a

change in blood volume due to a decrease in plasma.

Amytal decreased the plasma level of protein and glucose in the starved animal. In pairs of animals which had fasted for 1, 3, and 5 days it lowered the protein from 0.3 to 0.8 grams, and the blood sugar from 16 to 24 mgm. per cent.

Recovery from fasting was examined in the first experiment in a preliminary way. Five animals that had been fasted for 3 days were given food, and single animals were examined 2 and 3 days later, under ether. To our surprise, in both cases, the total protein was only 6.2 grams per cent, the glucose about 135 and the NPN about 33 mgm. per cent. A test with amytal lowered the total protein and glucose as usual. It thus appeared that after a 3 day fast, the provision of food for 3 days resulted in perhaps 50 per cent recovery. Two other animals tested 19 days later were normal in all respects. That the animals were eating considerably during this period, was demonstrated from their weight curves which showed at least a 10 gram response the first day food was restored.

In the second experiment, animals which had been fasted for 1, 2, 3, and 5 days were then provided with food, and 2 and 5 days later analyses were done. The chlorides were normal and require no comment. In the animals starved for 3 or more days, the A/G ratio rose to 2.2 or more; it was still elevated after 2 days of refeeding, but was normal after 5. The data for total protein, glucose and NPN are shown in Figure 4. It is apparent that fasting for 1 day requires more than 2 days for recovery, but probably not more than 3. Fasting for 2 or 3 days will require something like 5 days for recovery of the blood chemistries. The control weight is not reached for about a week.

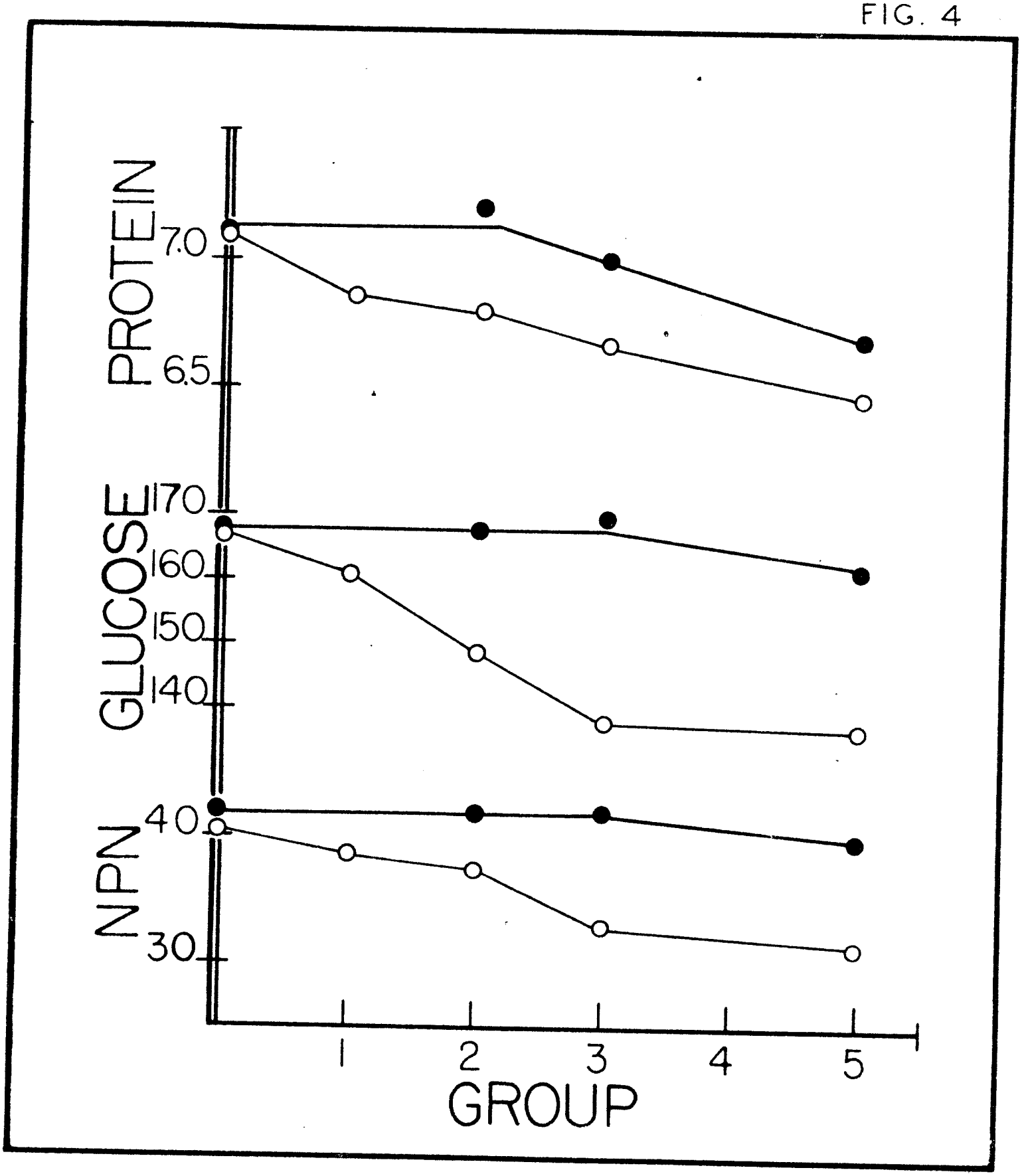
DISCUSSION

The choice of standardized conditions for bleeding experimental animals always introduces an arbitrary element into the experimental design. No doubt

Figure 4

Recovery from fasting. The group numbers indicate the number of days of fasting. The open circles record the plasma protein, NPN or glucose after 2 days of refeeding, the solid circles after 5 days. Each point is the average of two animals.

FIG. 4



the first requirement for any procedure is that it shall give consistent results. Another goal is simplicity.

The data presented in this paper indicate that unless some special requirement must be met (as in the study of liver glycogen), there is no advantage in starving the animals. As a matter of fact, starvation tends to complicate the experimental situation, especially since base line levels are not obtained in less than 48 hours.

As to anesthesia, it appears that ether is the anesthetic of choice. It is certainly the easiest to administer, and, under the conditions outlined here, has the shortest action and deranges the blood chemistries the least. While it is true that the plasma glucose is higher under ether than under barbiturates, the difference is no greater than is found in the excited, unanesthetized animal, and the determination is certainly valid for various comparative purposes.

It should be emphasized that the time factor is extremely important. Not only does the type of drug and its dose, but also the duration of its action determine the extent to which the "normal" levels will be shifted. The very rapid changes occurring under the barbiturates illustrate this point. No doubt the rapidity with which our cardiac punctures were done under ether prevented some of the major dislocations which occur with this anesthetic. Bonnycastle (4), for example, showed that ether and the barbiturates affect the blood, plasma, and interstitial fluid volumes markedly and in opposite directions. Under our conditions for the use of ether, the results match those obtained in animals which have recovered from barbiturate anesthesia, or are still mildly sedated.

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