ficially the islet structure seemed greatly restored, many of the regenerated cells were fibroblasts.

Experiments on Dogs. The amount of insulin in the pancreas of dogs was estimated at different times after the administration of 150 milligrams alloxan per kilogram. D 27 was given the full amount in a single injection. The other four animals received 4 equal doses at 15-minute intervals. Sections were taken from different parts of each pancreas and the remainder used for the insulin assay. The values in Table 2 were calculated as units per gram of total pancreas.

TABLE 2*

Dog number	Time after alloxan	Blood sugar	Insulin per gram of total pancreas
	(hours)	(mgms. per cent.)	(units)
D 21	0	80	2.5
D 22	2	340	3.5
D 23	4	195	2.2
D 24	8	45	3.2
D 25	24	278	none detectable
D 27	36	40	0.16

* The blood sugars reported in Table 2 are the ones obtained immediately before the termination of the experiment. Blood sugars, however, were determined at frequent intervals throughout the experiment on each dog. With the exception of D 27 there was the initial hyperglycemia reported by other workers. In D 24 and D 25 hypoglycemic levels were reached in 8 hours. D 27 showed no increase in blood sugar throughout the whole experiment and in 10 hours the blood sugar had fallen to 40 milligrams. The animal had convulsions which continued in spite of intravenous glucose until it was sacrificed.

The histological findings in the islet cells of the above dogs were as follows: in D 2 the capillary spaces were widened; the islet cells were not as regularly arranged as normally; some of the nuclei were pycnotic and many cells appeared shrunken. In D 23 pycnotic nuclei were more frequent and the cell cords were greatly disorganized. In D 24 most of the islet cells were necrotic with their nuclei failing to stain blue with hematoxylin. Small hemorrhages were seen in the necrotic tissue. In D 25 the islets were shrunken and difficult to detect. Their internal structure was disorganized and dead cells were seen in them. The smaller veins throughout the pancreas showed many marginal neutrophiles and the epithelium of many of the smaller ducts was vacuolated. In D 27 the islets seemed to be fewer and smaller than normal and there was considerable variation in the histological picture they presented. Few normal cells were seen in them.

The experiments on both the rats and dogs support the view that alloxan quickly destroys islet cells and that the insulin content of the pancreas does not fall substantially until some time after most of the islet cells are dead. The subsequent release of insulin which presumably lowers the blood sugar can not then be in the nature of a secretory phenomenon because it occurs when the islet cells are dead; it must, therefore, be a process wherein the insulin content of the islets is leached from the dead cells into the blood stream.

Further evidence that the hypoglycemic effect of alloxan is due primarily to its action on the pancreas rather than upon the liver or other tissues is provided by the following experiment in which alloxan failed to exert a hypoglycemic effect in animals containing little or no islet tissue.

Alloxan was given as described above to 2 depancreatized dogs, to 2 dogs that had been made diabetic by previous treatment with alloxan and to a normal dog. The blood sugars of the diabetic dogs were controlled by an injection of protamine zinc insulin given 18 hours previous to alloxan. In both types of diabetic dogs receiving alloxan there was no reduction in blood sugar. This is in direct contrast to the result in this and many other normal dogs in which hypoglycemia occurred in 5 to 6 hours after alloxan.

Summary and Conclusions. Experiments are reported in which insulin assays and histological studies were made on the pancreases of rats and dogs at different times after the administration of alloxan. It was found that the insulin content of the pancreas did not fall appreciably until after most islet cells were found by histological examination, to be dead. A further experiment showed that alloxan did not exert a hypoglycemic effect in depancreatized dogs and dogs previously made diabetic with alloxan.

These experiments indicate that alloxan causes hypoglycemia because it kills islet cells and so allows their insulin content to be leached out into the blood stream. It appears most unlikely that it exerts its hypoglycemic effect by stimulating islet cell secretion because most of the islet cells are dead when the hypoglycemic phase occurs.

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THE CREATINE AND CREATININE EXCRETION OF NORMAL ADULT MALES[1,2]

IT is the purpose of this report to subjoin the data on the creatine and creatinine excretion of 30 normal human subjects which were collected from our investigations in human amino acid deficiencies to the existing and overwhelming factual evidence which indicates

[1] From the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

[2] Aided by grants from the Rockefeller Foundation, E. Merck and Company, Eli Lilly and Company, E. R. Squibb and Sons.

a long overdue revision of the prevalent statements; that, in a given individual, the elimination of creatinine is constant from day to day and that creatine is not present in the urine of the normal adult male.

Although the general validity of Folin's Law of constant daily creatinine has been generally accepted the constancy which it asserts must be allowed to be something short of absolute, as is indicated by the data upon which it is based.[3] In an extended study on 33 normal male and female subjects on hospital fare, Wang[4] found fluctuations of 10 to 25 per cent. of the individual creatinine output to be of common occurrence. In our experiments with 30 normal subjects studied for 38 to 60 days under normal and experimental dietary conditions individual daily variations of 10 to 25 per cent. of the total creatinine were also observed. These observations would seem to indicate that the irregularities in creatinine excretion are greater than heretofore presumed and that variations in creatinine output are not necessarily indicative of inaccurate 24-hour collections.

The truly remarkable feature of the creatinine excretion is its relative constancy under tremendous variations in protein intake. In this we have been able to amply confirm the findings of Folin, for, in subjects whose protein intake was changed from 13 to 14 g N/day to 1.5 g N/day, the daily creatinine variation was found to be no greater than that observed at normal protein intake levels. It is noteworthy, in this connection, that Benedict[5] observed a creatinine variation of only 1.40 to 0.86 g/day in the 30-day fasting man which is not greater than that found in normal well-fed individuals.

The failure of the earlier workers to observe creatinuria in the normal adult male is difficult to explain except that it might have been due in part to the lack of adequate methods and equipment which will be discussed below. In Table 1 are compiled summaries of the data from the investigations of others and ourselves. It is clear from this evidence that creatinuria occurs in all age groups of males so far investigated and that the observed values fall well within those found for women. Hobson[6] found, moreover, that a high carbohydrate intake increased the creatinuria; whereas, the amount of physical exercise was without effect. In our studies the creatine and creatinine output were measured daily for periods of 38 to 60 days and absence of creatine from the urine was sporadic and never extended for more than a single day in any of the subjects studied. Since meats, milk and cereal proteins were excluded from these diets, the regimen is considered creatine poor. On the basis of these findings, creatinuria in the adult male can not be considered symptomatic of starvation or hyperthyroidism, unless it be in excess of the levels recorded in Table 1.

TABLE 1

CREATINURIA IN NORMAL ADULT MALES

Investigator	Age group studied	Age limit of creatinuria	No. of subjects studied	No. of subjects with creatinuria	Creatine excreted/day	Diet
	yrs.	yrs.			mg	
Folin and Denis[12]	8–17	17	3	3	90–150	Normal
Rose[13]	1–19	13	19	13	25.3/100cc	Normal
Light and Warren[14]	14–19	19	81	35	17–117	Normal
Taylor and Chew[10]	20–34	34	15	14	0–196	Normal
Djen and Platt[11]	21–58	58	148	108	0–428/1.	Chinese diet
Hobson[6]	19–30	30	96	97	92–1200	Normal; carbohydrate varied
Wang[4]	20–55	55	15	11	0–200	Normal
Authors	19–35	35	31	31*	0–796	Various
	19–35	35	14	14	184–352	Normal
	19–25	25	9	9	202–358	Vegetables, fat and carbohydrate
	19–25	25	8	8	17–796	Casein digest, fats, vegetables, carbohydrates
	19–23 (females)	23	4	4	61–354	

* Based on 850 individual determinations.

In another series of experiments on 4 adult males, we found that the ingestion of 6 to 10 gms arginine hydrochloride daily in addition to the normal diet for periods of one month or more failed to affect the creatinine or creatine levels in the urine.

EXPERIMENTAL

Collection of urine. Complete 24-hour specimens were collected in brown bottles containing 50 cc 15 per cent. HCl and 1 cc 10 per cent. alcoholic thymol and diluted to a uniform 2-liter volume (pH 4–5) before removing samples.

Creatinine Determinations. The method of Folin[7] was adapted to the Klett-Summerson photoelectric colorimeter. The color measurement was made with the S-54 filter and a solution containing 1.6102 mg/cc creatinine zinc chloride (C. P. Pfanstiehl, 23.3 per cent. N found) in 0.1 N HCl equivalent to 1 mgm/cc creatinine served as the standard. The color reaction conforms to Beer's Law from 0.5 to 3.0 mgm creatinine/100 cc. It was found that 1 cc samples of urine give suitable readings. Care was taken that the picric acid conformed to the standards of purity established by Folin and Doisy[8] (20 cc saturated picric acid solution diluted to 100 cc with water served as the reagent blank). A series of precision tests revealed the

[3] O. Folin, *Am. Jour. Physiol.*, 13: 45–117, 1905.
[4] E. Wang, *Acta Med. Scand.*, Suppl. 105, 1939.
[5] F. G. Benedict, "A Study of Prolonged Fasting," Carnegie Inst. of Washington, Washington, D. C., 1915.
[6] W. Hobson, *Biochem. Jour.*, 33: 1425, 1939.
[7] O. Folin, *Jour. Biol. Chem.*, 17: 469, 1914.

optical colorimeter technique to introduce an error of ± 10 per cent. in the final result.

Creatine Determination. The conversion of creatine to creatinine was accomplished by the autoclave method of Folin.[7] Inasmuch as the creatine value is obtained by difference of the preformed and total creatinine, it was considered advisable to check the amount of creatinine destroyed and the extent of creating conversion effected by this procedure. The decomposition of creatinine was studied by submitting 1 cc samples of the creatinine zinc chloride standard to the Folin autoclave technique for varying periods of time (Table 2). A similar experiment with creatinine (C. P. Pfanstiehl) indicated an equal amount of destruction. These colorimetric findings were checked with a gravimetric procedure in which the creatinine was isolated and weighed as the flavianate in samples before and after autoclaving with 3 cc 10 per cent. flavianic acid for 20 minutes at 121° C. Three 50 mg samples in 10 cc which were not autoclaved yielded an average 191.4 mg creatinine flavianate or 50.2 mg creatinine; three autoclaved samples yielded 178.4 mg creatinine flavianate or 47.1 mg creatinine. This indicates a 6 per cent. decomposition of creatinine under the conditions of the method.

The efficacy of the creatinine conversion was tested by autoclaving 1 cc samples of a 0.1 N HCl solution containing 1 mg/cc creatine (C. P. Pfanstiehl, 32.0 per cent. N found) with 20 cc saturated picric acid solution. The extent of conversion was estimated colorimetrically (Table 2). In our experiments with the 3-hour boiling procedure of Folin[9] 83.5 to 86 per cent. creatine was found as creatinine. Due to the destruction of creatinine, it is obviously not possible to realize a complete conversion of creatine by this method.

TABLE 2

THE DECOMPOSITION OF CREATININE AND CONVERSION OF CREATINE TO CREATININE BY AUTOCLAVING AT 121° C.

Autoclaving time minutes	Creatinine decomposition Per cent.	Creatine conversion to creatinine Per cent.
10	7	67.5
20	8	86.5
40	9	92.6
60	9	93.0

Since the creatine value for adult males is normally about 10 per cent. of the creatinine output, it is understandable that if a correction is not made for the 8 to 9 per cent. creatinine decomposition incurred a low or no creatine output will be observed. It was found convenient to effect this correction by obtaining the creatine value as the difference of the total creatinine and the preformed creatinine of 1 cc samples which were simultaneously autoclaved with and without picric acid. The value so obtained is further corrected for the conversion of creatine into creatinine by the factor 1.16. The final formula for calculating creatine becomes:

$$\text{Creatine mg/day} = \frac{R_1 - R_2}{R_3} \times 1.16 \times \text{urine volume of specimen}$$

Where R_1 = total creatinine reading of 1 cc urine autoclaved with 20 cc saturated picric acid.

R_2 = preformed creatinine reading of 1 cc urine autoclaved without picric acid.

R_3 = reading of 1 cc of autoclaved standard containing 1 mg creatinine in 0.1 N HCl.

A comparison of the creatine excretion of normal adult males from 23 to 25 years of age on normal diets as determined by the Folin method and our modification showed our values to be 30 to 50 per cent. higher.

Inasmuch as creatinuria in the adult male has been consistently observed by others[6, 10, 11] who used the original Folin procedure, our creatinuria findings can not be considered specifically due to our technique, but we feel that a more accurate result is attainable by our modification.

SUMMARY

(1) It has been observed that the creatinine excretion of males and females is not an absolute value for the individual and is not influenced appreciably by the level of protein intake.

(2) Creatinuria in the adult male is a normal process and not a feminine or prepuberal male characteristic.

(3) A simple modification of the Folin method for determining creatine is described which reveals considerable quantities of creatine that may be present in urine but not detectable by the Folin technique.

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CORN OIL AND BUTTERFAT ESSENTIALLY EQUAL IN GROWTH-PROMOTING VALUE

THE Committee on Fats of the Food and Nutrition Board, National Research Council, has recently published a report on margarine.[1] In an evaluation of the relative nutritive value of different fats it comes to the conclusion that: "There is not complete agreement in the results from different laboratories as to the comparative value of different fats fed at the same level. Furthermore, with repeated experiments the results are not always the same in a given laboratory. Differences between fats will be found at one time which are statistically significant but which are not reproducible at another time with a new group of rats and a new batch of the same kind of fat."

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[1] Reprint and Circular Series, No. 118. August, 1943.