penicillin depends on the dose used. A high penicillin blood level was obtained with 3,-600,000 units of procaine penicillin. A blood serum penicillin level very superior to the minimum effective level was obtained with implantation of 1,000,000 to 2,000,000 units of procaine penicillin.

Summary. Tablets of procaine penicillin implanted in the subcutaneous or muscular tissue provide a safe procedure for prolonging the penicillin serum blood level for several days.

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Biochemical Studies on the Alligator. (17627)

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In the course of a search for a new experimental animal for certain biochemical studies it was decided to investigate the suitability of the large North American alligator, *A. mississipiensis.* Some preliminary analyses revealed a striking discrepancy between some of the results obtained in the present study and those reported earlier by Hopping(1), Dill and Edwards(2), and Austin *et al.*(3). Most of the work that had been done on the alligator was repeated by the present authors and many additional studies were conducted. This paper deals with the results obtained on the fasting alligator kept under laboratory conditions. The effect of feeding, temperature, and seasonal variation on the composition of the blood and urine is quite marked and will not be reported here.

This study includes the data obtained from 65 alligators of undetermined sex. Twelve were 3-4 years old and weighed from 1.20-7.37 kg; 5 were 2-3 years old and weighed 0.79-2.03 kg; and 48 were about one year old and weighed from 48-182 g. The animals were kept at an average temperature of about 26°C in monel metal cadaver tanks which were filled to a depth of about one inch with water. They were fed fresh skinned rats from our colony. Although the alligators were avid feeders in the early spring and summer they tapered off in October and November and ate very little in December, January, and February. Under these conditions the animals doubled their weight in a 12 month period.

Blood was withdrawn from the tip of the tail for glucose determinations. All other determinations were done on blood obtained conveniently by cardiac puncture by the method suggested by Hopping(1). Amounts up to 10 ml were drawn by this procedure without injury to the alligator. The electrolyte studies were conducted on blood kept under mineral oil using potassium or ammonium oxalate as the anticoagulant. Urine was obtained by cloacal catheterization using a number 14F catheter.

Analytical Methods. Ammonia and urea of the blood and urine were determined by the method of Van Slyke and Cullen(4), uric acid by the method of Folin(5) and Brown(6), creatinine by the method of Folin and Wu(7), and protein and N.P.N. by the micro-Kjeldahl method of Howe(8). The pH determinations were done on a Beckman Model G pH meter with the Beckman glass electrode designed for blood analyses. Chlorides were determined

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5. Folin, O., *J. Biol. Chem.*, 1934, v106, 311.
6. Brown, H., *J. Biol. Chem.*, 1945, v158, 601.
7. Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, v38, 81.
8. Howe, P. E., *J. Biol. Chem.*, 1921, v49, 109.

TABLE I.
Blood Studies.

	No. det'ns	Avg	Range
Cell count, mm^3	29	481000	331000-676000
White Cells, %	33	6.84	4.7-10.8
H'crit, %	29	21.8	16-29
M.C.V., μ^3	29	472	361-562
Sed. Rate, mm/hr	33	5	2-7
Hb., %	15	7.0	5.0-9.2

by the method of Schales and Schales(9) and the bicarbonates with the Van Slyke(10) manometric apparatus. The Beckman flame photometer was used to determine sodium, potassium, and magnesium. Serum calcium was done by the Clark-Collip(11) modification of the Kramer-Tisdall(12,13,14) method, urinary and fecal calcium by flame photometry, and serum phosphorus by the method of Kuttner and Cohen(15). Blood glucose was determined by the method of Folin and Wu(16) and liver glycogen by the method of Good *et al.*(17). Total blood volume was estimated by the use of Evans Blue dye(18). The Klett-Summerson Photoelectric colorimeter was used for the colorimetric determinations.

Results. Table I presents a summary of the results of the general blood studies which were conducted throughout the year. For some reason our values for hematocrit, cell count, and mean corpuscular volume are quite dissimilar from those reported by Hopping. Calculation of the M.C.V. from the data of Hopping gives a value of 160 cubic micra as compared with the 472 cubic micra we found. It is possible that the reported cell counts were inaccurate since the dimensions she reported for the individual cell are in keeping with our own observations. The blood volume was determined on one alligator weighing 2 kg and was found to be 4.2% of the body weight. No other blood volume determinations were conducted since the dye colored the blood for a period of many weeks which made it unfit for colorimetric determinations of its constituents.

Hundreds of glucose determinations were done at various times and at every month throughout the year. There was considerable seasonal variation in the results obtained. The "average" figure of 99 mg % includes individual values for about 25 alligators for each month of the year. For the 300 analyses reported here the highest fasting level found was 205 mg% (August) and the lowest was 21 mg% (November). Liver glycogen analyses were done on 2 animals; one had a glycogen content of 9.62% of the net weight of the liver and the other 8.00% (July).

The blood N.P.N. and ammonia determinations were made during the summer, fall, and winter months. The values obtained in this study (Tables II and III) are much higher than those previously reported(1,3). It is apparent that ammonia is not only present in the blood in considerable amounts in these alligators but is indeed the chief nitrogenous constituent of both blood and urine. It is improbable that any error was made in these determinations since ammonia was determined on all blood samples immediately after withdrawing blood and since the N.P.N. as determined by the Kjeldahl method was also much higher than that found by Hopping and Austin *et al.* for their alligators. The failure of Hopping to find

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TABLE II.
Blood Nitrogenous Constituents.

		No. det'ns	Avg	Range
Blood	NH_3, mg%	27	61	18-98
ʼʼ	Urea ʼʼ	10	0	—
ʼʼ	Uric Acid, mg%	42	2.25	0.96-4.23
ʼʼ	Creatinine ʼʼ	27	1.80	0.40-3.12
Serum	NPN, mg%	22	90.8	57.4-132.8
ʼʼ	Protein, %	14	5.02	3.37-6.02

TABLE III.
Urine Nitrogenous Constituents.

	No. det'ns	Avg	Range
NH_3, mg%	23	216	116-262
Urea, ʼʼ	9	tr.?	0-tr.?
Uric Acid, mg%	20	101	30-208
Creatinine, ʼʼ	19	3.5	2.2-6.1
Total N, ʼʼ	6	273	208-334

TABLE IV.
Blood Electrolytes.

		No. det'ns	Avg	Range
Blood	pH	58	7.30	6.87-7.66
ʼʼ	Cl, meq/L	18	96	80-107
ʼʼ	K, ʼʼ	4	32.8	23.1-44.9
Plasma	Na, ʼʼ	29	154	146-162
ʼʼ	K, ʼʼ	10	4.2	2.6-6.4
ʼʼ	HCO_3 ʼʼ	18	21.9	14.3-39.3*
ʼʼ	Cl, ʼʼ	29	107	83-128
Serum	Ca, mg%	12	10.2	9.4-11.0
ʼʼ	P, ʼʼ	11	3.39	2.86-3.85

* One alligator maintained a persistent alkalosis over a period of several months. It is improbable that any of the other animals would ever approach a fasting HCO_3^- level of 39.3.

TABLE V.
Urine Electrolytes.

	No. det'ns	Avg	Range
pH	62	7.72	7.18-8.02
$\bar{H}CO_3$ meq/L	13	105	77.6-125
Cl, ʼʼ	30	tr.	0-tr.
Na, ʼʼ*	9	3.19	Pooled
K, ʼʼ*	9	11.1	ʼʼ
Ca, ʼʼ*	9	13.3	ʼʼ
Mg, ʼʼ*	9	1.0	ʼʼ

* Done on ashed specimens.

creatinine in the blood is difficult to explain. Although the urinary ammonia is fairly consistent at a level of about 0.1 M. we were unable to detect any measurable quantity of urea in the blood or urine.

Blood and urine electrolyte studies were conducted during the summer months and the results are summarized in Tables IV and V. The principal cation of the urine is NH_4^+ and the principal anion is $\bar{H}CO_3$. The kidney of the fasting alligator seems to conserve both sodium and chlorine to a considerable extent since neither of these ions was present in any quantity in the urine although both were present in the blood in amounts similar to those reported for man. The alligator is apparently exceptionally well adapted for conserving sodium chloride since he loses very little in the feces (Table VI).

The pH of the urine of the fasting alligator

TABLE VI.
Composition of Fecal Ash.

	Dry wt, g	Ash, g	Cl	CO_3, %	Na, %	K, %	Ca, %	Mg, %
Sample 1	14.42	9.38	tr.	51.47	.416	.037	30.92	.427
" 2	0.97	0.76	0	—	.215	.026	32.26	.827

TABLE VII.
Composition of Alligator Ash.

Alligator, No.	Wt, g	Dry wt, g	H_2O, %	Ash, g	Cl, mg	Na, mg	K, mg	Ca, mg	Mg, mg
1	71.6	12.9	81.9	2.459	42.25	112.5	130.2	290	16.4
2	91.5	20.7	77.4	3.001	120.20	155.0	161.0	565	9.3
3	68.0	14.5	78.7	2.319	89.25	106.6	111.9	327	18.8

is always more alkaline than that of the blood. The blood pH shows a remarkable variation from animal to animal and even in the same animal at different times of the day. The resting pH is very difficult to determine since hyperventilation occurs when the animal is excited, and consequently it was necessary to do a large number of determinations on blood and urine because of the great variations found. Analyses of 2 specimens of fecal ash of alligators on a diet of fresh skinned rats are reported in Table VI. The dried feces are gray in color and contain almost no organic matter. The principal component seems to be $Ca(HCO_3)_2$. Three small alligators were also ashed in an electric furnace and the results are recorded in Table VII.

Comments. Neither Hopping(1), Dill and Edwards(2), nor Austin *et al.*(3), used alligators which were known to have fasted before their studies were made. It should be emphasized here that all chloride, bicarbonate, pH, and sodium determinations must be conducted on alligators which have fasted for at least 3 days because of the great magnitude of the "alkaline tide" with all of its accompanying changes in blood electrolytes(19).

It has been our experience that alligators make excellent laboratory animals for many types of experiments. They are hardy, easy to feed, require much less laboratory space than mammalian forms of comparable size, and, in spite of popular opinion to the contrary, they are easy to handle.

Summary. The following determinations have been done on fasting alligators: blood cell count, % white cells, hematocrit, M.C.V., sedimentation rate, hemoglobin, blood vol., blood glucose, liver glycogen, blood and urine ammonia, urea, uric acid, creatinine, serum proteins and N.P.N., blood and urine pH, chloride, sodium, potassium, bicarbonate, and calcium, urine magnesium, and the electrolytes of the whole ashed alligator and alligator feces.

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19. Unpublished observations.

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