vestigate this point a study was run in which known malignant urine extracts were injected into normal and hypophysectomized rats. The results obtained are listed in Table 1. In this study, 39 out of 40 known malignant urine extracts gave a positive biological test two days after injection. In 32 urines of unknown diagnosis the test was positive in 6 malignancies and negative in 26 nonmalignancies. All urine extracts from normal individuals and other nonmalignant cases were negative.

The following views are supported by the results:

(1) Malignant urine extracts lose about 60 per cent of their ability to give the test after standing in the ice chest for four months.

(2) The biological test is *negative* in the hypophysectomized rat.

(3) *All malignant urines so far tested by ourselves (with one exception) and others* contain a substance, X, which is protein-sterol or sterol in nature and which stimulates the rat pituitary to produce increased amounts of another substance, Y, possibly a gonadotropic hormone, which is then responsible for the biological test for human malignancy.

The idea given in (*3*) is confirmed by Gurchot, Krebs, and Krebs (*2*), who stated: "The experiments herein described indicate that normal human pregnancy trophoblast (from human placental tissue), when grown in the anterior chamber of the eyes of rabbits, behaves like a malignant tumor grown in a similar medium. Our experiments, as well as those of others, suggest that all malignant tumors, regardless of their protean character, are fundamentally trophoblastic."

References

1. Carratala, A. T. *Bol. Inst. Med. Exp.*, 1944, No. 64, 204; Roffo, A. H. *Bol. Inst. Med. Exp.*, 1944, No. 65, 419; Krebs, E. T., and Gurchot, C. *Science*, 1946, **104**, 302.
2. Gurchot, C., Krebs, E. T., Jr., and Krebs, E. T. *Surg. Gynec. Obstet.*, 1947, **84**, 301.
3. Schabad, L. M. *C. R. Soc. Biol. Paris*, 1937, **124**, 213; 1937, **126**, 1180; Steiner, P. E. *Cancer Res.*, 1942, **2**, 425; 1943, **3**, 485.

Serum Phosphatases and Alloxan Diabetes[1]

Max M. Cantor, Jules Tuba, and Persis A. Capsey

Department of Biochemistry, University of Alberta, Edmonton, Canada

Alloxan diabetes was produced in adult male albino rats by a single subcutaneous injection of 16 mg. of alloxan monohydrate for each 100 grams of body weight. The animals were housed in metal cages, 6 to a cage, and fed Purina Fox Checkers with tap water *ad lib.* Of 61 animals used, 6 per cent were unaffected by the treatment; 10 per cent recovered from an early hyperglycemia; 20 per cent developed hyperglycemia and died within three days. The remaining 64 per cent survived for long periods and exhibited hyperglycemia, glycosuria, lipemia, and ketonuria. Polyuria and polydipsia were marked, and there was progressive weight loss to the extent of 50 per cent of the original weight in three weeks.

Specimens of tail blood were obtained periodically for the estimation of blood glucose, serum inorganic phosphorus, and acid and alkaline phosphatases. Blood sugar was estimated by the micromethod of Reinecke (*5*). Serum phosphorus and phosphatases were determined by the method of Shinowara, *et al.* (*6*) as modified by Gould and Schwachman (*3*). Acid and alkaline phosphatases were estimated at pH 5.3 and pH 9.3, respectively. The normal mean values, previously determined in fasted animals, were: blood sugar, 117 mg./100 ml.; serum inorganic phosphorus, 9.0 mg./100 ml.; serum acid phosphatase, 3.2 units/100 ml.; and serum alkaline phosphatase, 113 units/100 ml.

Six animals were used for each experiment and the results averaged. Table 1 presents the findings in a representative group.

There is a rapid, progressive rise in blood sugar. This exceeds 400 mg. per cent at the end of two days and is maintained thereafter with only slight fluctuations. The initial transient rise in blood sugar in rabbits noted by some observers (*4*) and attributed to the release of adrenalin was not observed in the present experiment. In our subsequent experiments, not reported here, in which the dosage of alloxan was greater, such an early transient blood sugar rise was observed. The acid phosphatase falls quickly to about 25 per cent of the initial level and returns to normal in three days. Alkaline phosphatase shows a similar initial decline which is restored within 12 hours. This is followed by a progressive increase over the next two weeks, when values in excess of three times the normal level are reached. Serum inorganic phosphorus is not greatly altered, but nearly all values found after the injection of alloxan are greater than the initial levels.

TABLE 1

Effect of Alloxan on Blood Sugar, Serum Phosphatases, and Inorganic Phosphorus

Time after administration of alloxan	Blood sugar (mg./100 ml.)	Serum alkaline phosphatase (units/100 ml.)	Serum acid phosphatase (units/100 ml.)	Inorganic phosphorus (mg./100 ml.)
0 hrs.	123	109	3.6	8.3
2 "	166	—	—	—
3 "	216	56	0.93	—
6 "	—	64	1.0	10.9
9 "	250	89	1.3	9.9
12 "	310	105	1.6	9.1
24 "	380	125	1.7	8.4
2 days	464	154	1.5	8.6
3 "	440	179	3.7	8.9
4 "	425	205	4.2	9.8
5 "	426	223	4.2	10.5
7 "	358	177	4.1	9.4
8 "	412	250	4.0	9.9
14 "	444	365	4.6	9.0
21 "	420	371	3.7	9.3
42 "	437	380	4.4	10.1

The hyperglycemia and ketonuria which develop are easily controlled with insulin. A single subcutaneous injection of 0.4 units of crystalline zinc insulin restores the blood sugar to within normal levels in three hours. This is accompanied by a 30 per cent decline in both acid and alkaline phosphatases. At the end of 24 hours all values are restored to the diabetic levels. In order that the effect of insulin could be observed over

[1] This work is part of Project M.P. 80, conducted with the assistance of a grant to M. M. Cantor from the Division of Medical Research, National Research Council, Ottawa.

a longer period, three doses of 0.4 units of insulin were injected at three-hour intervals. The averaged results in four animals are presented in Table 2.

Blood specimens obtained 90 minutes after the third injection of insulin show hypoglycemia, reduction of acid phosphatase to 25 per cent of normal, and reduction of the alkaline enzyme to within normal limits. Serum inorganic phosphorus is reduced.

TABLE 2

EFFECT OF THREE DOSES OF 0.4 UNITS OF INSULIN AT THREE-HOUR INTERVALS ON BLOOD SUGAR, SERUM PHOSPHATASES, AND INORGANIC PHOSPHORUS IN ALLOXAN-DIABETIC RATS

Time after injection of insulin	Blood sugar (mg./100 ml.)	Serum alkaline phosphatase (units/100 ml.)	Serum acid phosphatase (units/100 ml.)	Serum inorganic phosphorus (mg./100 ml.)
0 hrs.	425	384	3.9	11.8
1st dose				
1½ hrs.	150	346	3.1	11.1
3 "	154	284	2.3	11.5
2nd dose				
1½ hrs.	77	236	2.0	11.5
3 "	101	197	2.4	9.2
3rd dose				
1½ hrs.	51	161	1.0	10.2
3 "	122	132	1.0	9.8
9 "	410	206	2.3	7.4

Our findings indicate that the development of alloxan diabetes in rats is accompanied by an increase in serum alkaline phosphatase activity and that the administration of insulin produces a decrease in the activity of both the acid and alkaline enzymes. The initial decline in phosphatase activity following the injection of alloxan simulates that produced by the injection of insulin and is attributed to the release of insulin stores in the pancreas. When this supply is exhausted, alkaline phosphatase activity increases and remains elevated, the increase being parallel to the elevation in blood sugar. Both are reduced, again in parallel fashion, by the administration of insulin.

We have been unable to demonstrate any great increase in serum inorganic phosphorus following the administration of alloxan, although nearly all the values found were in excess of the preinjection level, and a few were greatly increased. Following the administration of insulin in the alloxan diabetic animal, reduction in the level of inorganic phosphorus was demonstrated. These findings are in accord with the well-known effect of insulin both in the diabetic and normal organisms (*1, 2*) and suggest that these alterations reflect changes in phosphatase activity.

References

1. BOLLIGER, A., and HARTMAN, F. W. *J. biol. Chem.*, 1925, **64**, 91.
2. CHAIKOFF, I. L., MACLEOD, J. J. R., and MARKOWITZ, J. *Amer. J. Physiol.*, 1925, **74**, 36.
3. GOULD, B. S., and SCHWACHMAN, H. *J. biol. Chem.*, 1943, **151**, 439.
4. JACOBS, H. R. *Proc. Soc. exp. Biol. Med.*, 1937-38, **37**, 407.
5. REINECKE, R. M. *J. biol. Chem.*, 1942, **143**, 351.
6. SHINOWARA, G. Y., JONES, L. M., and REINHART, H. L. *J. biol. Chem.*, 1942, **142**, 921.

The pH Stability of Viruses of Newcastle Disease and Fowl Plague[1]

H. E. MOSES

Purdue University Agricultural Experiment Station

C. A. BRANDLY

Department of Veterinary Science, University of Wisconsin

E. ELIZABETH JONES

Wellesley College, Wellesley, Massachusetts

The recognition (*2*) of the immunologic identity of the causative agent of avian pneumoencephalitis with that of Newcastle disease revealed the existence of a serious threat to the poultry industry of the United States. The clinical features of the American form of the disease have allayed suspicion of its identity with the classical, hitherto highly virulent Newcastle disease (*1*). The differences between the American and the classical forms may result from the noted marked pneumo- and neurotropism of the virus isolated in this country and the pronounced enterotropism of a European strain of the virus (*7*). In Europe and elsewhere, Newcastle disease has been mistaken for possible forms of fowl plague and vice versa, and immunologic evidence has been obtained to support both dissimilarity (*5*) and similarity (*8*) of these diseases. However, recent immunologic and other comparative studies have provided strong support for the unity of each disease (*4*). The importance of these two diseases, the one a real, and the other a potential, threat to the poultry industry of this country, prompted studies to obtain further information on the relationship of the American and European Newcastle disease viruses and of these, in turn, to the virus of fowl plague.

An evaluation by Doyle (*5*) of the effect of marked acidity and alkalinity on the Newcastle virus infectivity indicated greater resistance to the H- than to the OH-ions. The results of Pyl (*10*) in the case of the fowl plague virus showed that maximal stability of the infectivity occurred between pH 6.3 and 9.1 and that the virus was destroyed immediately at pH 4 and 12.7. This pattern was similar for brain and blood virus of chickens infected with the Brescia and Bologna strains of plague virus. These results and those for other viruses (*6, 10*) suggested or showed rather distinctive pH stability patterns.

Evaluations of the pH stability were made on four strains of the Newcastle disease virus, including the Hertfordshire strain (H) of English origin and three strains (11914, RO, and C) isolated from cases of "pneumoencephalitis" in California and provided by J. R. Beach, and on the Dutch East Indies strain of the fowl plague virus. A variant virus (Strain 4395), isolated from the plague virus in the course of other procedures (*9*), was subjected to similar study. The source of the virus used in all tests was the allanto-amnionic fluid of embryonated chicken eggs which had been infected with the respective viruses after 10–12 days of incubation. These materials were admixed in the ratio of 1:99, or, in a few tests, 1:9, with portions of a buffer solution (*3*) having pH values of from 2 to

[1] This work was done as a part of a research project conducted under the direction of a War Department Commission consisting of: Brig. Gen. R. A. Kelser, U. S. Army; B. E. Dyer, U. S. Public Health Service; H. W. Schoening, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture; and E. B. Fred, University of Wisconsin.