TECHNICAL PAPERS

Effect of Intraperitoneal Injection of Malignant Urine Extracts in Normal and Hypophysectomized Rats

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Lipid extracts of normal liver tissue and those from histologically normal liver and other tissues from malignant patients, when injected into rats and mice, cause the appearance of tumors in a number of the animals (*3*). The active factor resides in the nonsaponifiable sterol fraction.

Some time ago we began a study of alcohol-ether extracts of known malignant urines with the hope of producing tumors in rats receiving 2 cc. of these extracts, equivalent to 100 cc. of original urine. It was found, however, that 2 to 4 days after injection of the extracts there was an enlargement in the spleen and/or gonads. In 39 out of 40 known malignant extracts the decrease in the body weight:spleen and body weight:gonad ratios ranged from 20 to 80 per cent below these same ratios in the control rats with no injection. This constitutes a biological test of malignancy. In 26 normal urine extracts the test was negative (a decrease of not more than 20 per cent from the controls). The average per cent increase in weight of the organs in these 40 cases over that in the controls was as follows: spleen, 39; male gonads, 55; and female gonads, 72. There was intense hyperemia in the spleen and hyperplasia and intense spermatogenesis in the testes.

In 32 urines from patients whose diagnosis was unknown to us at the time the tests were made, there were 6 positive tests, one each from cancer of the breast, rectum, stomach, prostate, colon, and a malignant melanoma. In the remaining 26 cases all were normal or nonmalignant, and the tests in all were negative. These results confirm those of Carratala, Roffo, and Krebs and Gurchot (*1*) in this connection.

The malignant extracts gradually lose their potency if allowed to stand some time in the ice chest. The average decrease in the R/S ratios in fresh extracts was 47 per cent compared to only 17 per cent at the end of four months. These values for the R/G ratios were 61 and 24 per cent.

We believe that a substance, X, which is present in the blood and urine of all malignant patients so far tested, is either a sterol or a sterol-protein complex. Roffo, and Krebs and Gurchot, further stated that a gonadotropic hormone was responsible for the results of the biological test. In order to in-

TABLE 1

Normal rats								Hypophysectomized rats								Age of extract (mos.)
Ext. No.	Rat (grams)	Spleen (mg.)	Gonads (mg.)	R/S	R/G	Decrease (%) R/S	Decrease (%) R/G	Rat (grams)	Spleen (mg.)	Gonads (mg.)	R/S	R/G	Decrease (%) R/S	Decrease (%) R/G	Clinical diagnosis Ca of:	
C	87	267	90	326	966			69	180	282	383	245				
21	85	535	252	158	337	52	65	69	206	292	335	236	13	4	Bronchiogenic Ca	1
C	104	966	1,403	107	74			104	966	1,403	107	74				
21	95	1,073	1,903	89	50	17	32	98	941	1,094	104	90	0	0	Bronchiogenic Ca	4
C	115	868	1,105	132	104			69	180	282	383	245				
28	124	900	2,060	137	60	0	42	80	224	197	357	406	7	0	Breast	1
C	112	1,046	1,501	107	75			112	1,046	1,501	107	75				
28	132	1,216	2,321	108	57	0	24	101	832	1,350	121	74	0	0	Breast	4
C	97	333	87	291	1,114			109	850	1,395	128	78				
34	89	669	335	133	266	54	76	110	785	1,415	140	78	0	0	Prostate	1
C	95	903	1,381	105	69			95	903	1,381	105	69				
34	127	1,365	1,900	93	66	11	0	98	510	1,384	192	71	0	0	Prostate	4
C	107	483	200	221	535			111	863	1,175	128	94			Mediastinum	
37	107	748	530	143	201	35	62	123	915	1,410	134	87	0	7	(Mtas to spine)	1
C	81	680	1,190	119	68			81	680	1,190	119	68			Mediastinum	
37	109	1,160	1,875	93	58	22	15	83	660	946	126	88	0	0	(Mtas to spine)	4

[1] The authors wish to thank F. C. Koch, director of the Armour Laboratories, for the suggestion to use hypophysectomized rats and for instruction in the use of the Ingle technique of hypophysectomy.

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."

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Serum Phosphatases and Alloxan Diabetes[1]

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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

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