creased absorption of the cholesterol and that a considerable portion of this cholesterol was retained. The fact that the animals eating the linoleic acid excreted the least amount of 3-β hydroxy sterols and Lieberman-Burchard chromogens and had the highest concentration of cholesterol in the liver suggests that linoleic acid was the more effective of the two lipids in promoting cholesterol absorption.

Summary. Adding fat to the diet of the rabbit which contains cholesterol increased serum cholesterol. The increased serum cholesterol was associated with increased aortic tissue cholesterol. It is proposed that addition of the lipid to the diet increased absorption and retention of cholesterol and this intensified the cholesterol atherosis.

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In vivo Tumor Inhibitory Activity of Normal Human Serum.* (26079)

ERNEST P. NOBLE AND AUSTIN S. WEISBERGER
Department of Medicine and Biochemistry, Western Reserve University, Cleveland, Ohio

During the past few years increasing interest has been focused on the effect of serum and its components on normal and malignant animal cells. Willheim *et al.*(1,2) have shown that normal human serum is able to digest Ehrlich ascites and other tumor cells. Serum obtained from schizophrenic patients was shown by Federoff(3) to be toxic to strain L cells. Bolande and co-workers(4,5), have demonstrated cytotoxic action of serum for HeLa cells, U-12 cells of human origin, Ehrlich ascites and Sarcoma 180 cells. Björklund(6) has presented evidence for presence of a cytotoxic factor in normal serum coupled to an inhibitor capable of rapid and complete destruction of human tumors *in vitro*. The studies presented here, initiated 3 years ago, deal with comparisons of *in vivo* tumor inhibitory activity of sera from normal individuals and sera from patients with diseased states on Sarcoma 180 cells. It was found that normal human serum exerted a greater inhibitory effect on Sarcoma 180 ascites tumor growth than sera from patients with leukemia and related diseases. Attempts were also made at partial isolation and characterization of the normal human serum components toxic to the tumor cells.

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Materials and methods. Donor CFW mice with Sarcoma 180 tumor in the ascitic form were sacrificed under ether anaesthesia, 6-8 ml of non-hemorrhagic peritoneal fluid was withdrawn and immediately placed in 1.0 ml saline and 0.2 ml heparin (1000 USP units/ml). A cell count was made on an aliquot of the tumor suspension, and the remainder diluted with cold pH 7.4 isotonic saline so that each inoculum of 1 ml contained 2 x 10^6 cells.

Blood was withdrawn from healthy subjects and from patients prior to initiation of therapy. Rabbit, guinea pig, mouse and rat blood were obtained by cardiac puncture; and bovine, porcine, calf and lamb blood collected from the jugular vein. Defibrination was immediately carried out by agitating blood with glass beads and the cellular elements centrifuged at 3000 g in the cold. The supernatant was aspirated and frozen at −70°C. All subsequent handling of the sera and their fractions was carried out rapidly between 0 and 3°C.

A 90% saturated ammonium sulfate solution of normal human serum (NHS) was prepared by addition of solid ammonium sulfate,

TABLE I. Effect of Various Sera and Sera Fractions on Survival of Mice Inoculated with Sarcoma 180 Cells.

Inoculum	No. of sera tested	No. of animals tested	% mice surviving 17 days	% mice surviving 120 days
Tumor + isotonic saline	—	260	0	0
" + normal human serum	13	250	79 ± 12*	25 ± 13
" + 90% $(NH_4)_2SO_4$ precipitate of normal human serum	10	150	58 ± 13	25 ± 10
" + rabbit serum	3	45	16 ± 15	0
" + guinea pig serum	2	24	0	0
" + mouse "	1	15	0	0
" + rat "	2	32	0	0
" + bovine "	3	50	8 ± 10	0
" + porcine "	2	48	8 ± 8	0
" + calf "	2	48	8 ± 8	0
" + lamb "	2	50	4 ± 4	0
" + schizophrenic serum	4	60	88 ± 19	27 ± 15
" + serum from patients with blood dyscrasias	8	120	28 ± 14	0

* Mean ± S.D.

over a period of 10 minutes, to gently stirred serum. pH of the mixture was maintained between 6.4 and 7.5 with N NH_4OH throughout addition of the solid. After additional 5 minutes of stirring the resultant precipitate was centrifuged for 30 minutes at 10,000 g. The precipitate was dissolved and made up to its original serum volume with pH 7.4 isotonic saline and dialyzed for 18 hours against 20 times its volume of isotonic saline.

Swiss mice weighing 20-25 g each were given inoculations intraperitoneally, first of 1 ml tumor suspension containing 2 x 10^6 cells, followed directly with 2 ml of cold pH 7.4 serum or its fraction. A control group with each mouse receiving tumor and isotonic saline was run concurrently with each experiment. Groups of 15-20 mice were used for each test run and a similar number were used as a control. Gain in weight and length of survival were taken daily as an index of the amount of ascites formed and degree of malignancy induced. All dead mice were autopsied for presence of tumor cells and only those mice that had tumor were scored as tumor deaths.

Results. All control mice died in less than 17 days after injection of tumor cells and saline, whereas animals that received tumor cells and sera from healthy subjects were well protected at 17 days and one quarter of the original population lived for more than 120 days (Table I). The property of NHS for inhibiting tumor growth was found to be heat-labile in that when NHS was heated to 60° for 30 minutes all animals receiving this solution and tumor cells developed tumors. Most were dead at 17 days and none survived more than 120 days. Furthermore, tumor inhibitory activity of NHS was found to be non-dialyzable, precipitated in the euglobulin fraction, and its activity was found to be independent of Ca^{++} and Mg^{++}. In addition, between the pH range tested, 5-9, serum was found to exert its tumor inhibitory action. Finally, in contrast to NHS, sera of rabbit, guinea pig, mouse, rat, bovine, porcine, calf and lamb failed to protect mice against tumor development (Table I).

The precipitate obtained from NHS saturated with 90% ammonium sulfate gave mice a degree of protection against tumor about equivalent to that of whole NHS (Table I). However when 0-40, 40-50, 50-60. 60-70, and 70-90% saturated ammonium sulfate subfractions were prepared, in most experiments, a low tumor inhibitory activity was recovered in these fractions.

The abnormal sera group consisted of clinically diagnosed but untreated cases of schizophrenia and patients with various malignant diseases. The latter group was composed of 2 patients with acute leukemia, 2 with chronic myeloid leukemia, one with polycythemia vera and 3 with chronic lymphocytic leukemia. The results (Table I) indicate

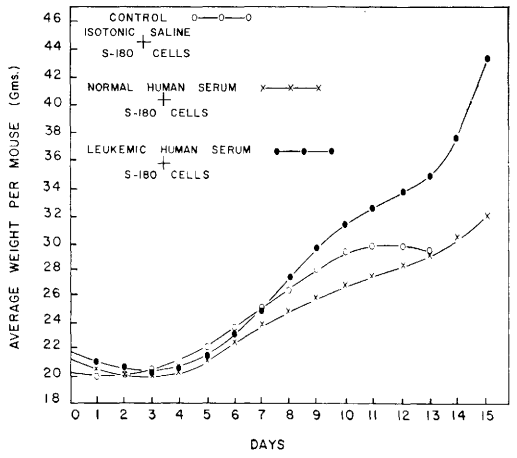

FIG. 1. Effect of saline, normal human serum and leukemic serum on growth of Sarcoma 180 ascites tumor cells in Swiss mice. Inoculation of a group of 15-20 mice with 2×10^6 Sarcoma 180 ascites tumor cells and 2 ml saline/mouse (control) resulted in rapid growth of tumor and death of all animals in 13 days. Substituting 2 ml normal human serum for saline resulted in marked inhibition of tumor development. No such inhibition was, however, observed when leukemic serum was used.

that schizophrenic serum, like NHS, also possesses *in vivo* tumor inhibitory activity. When mice, on the other hand, received an inoculum of Sarcoma 180 tumor cells and serum from patients with blood dyscrasias, the percent of animals surviving 17 days averaged lower than those given tumor cells and NHS. Furthermore, none of the mice that received tumor cells and serum from patients with malignant diseases survived for more than 120 days. Rate of tumor growth in mice receiving the latter serum was more rapid than in animals given NHS (Fig. 1).

Discussion. Although the advantages of an *in vitro* test system are numerous, the final test of cytotoxic activity of an agent must be elicited in an *in vivo* system. The present studies show, indeed, that NHS contains non-specific components inhibitory to Sarcoma 180 proliferation *in vivo*, whereas such activity appears to be low or lacking in serum of individuals with various blood dyscrasias.

It is a common clinical observation that patients with leukemia receiving blood transfusions exhibit a transient fall in leukocyte count as well as a general improvement in well being. That NHS may contain certain factors capable of destroying atypical cells *in vivo* is also suggested by Southam and Pillemer(7) who found that normal subjects rejected cancer cell implants whereas in patients with advanced carcinoma, growth of the implanted cells occurred at almost all sites.

The tumor inhibitory activity of NHS described here does not appear to be properdin (8) since no Mg^{++} or other divalent cations were required for such activity. Furthermore, the serum of animals other than man, known also to contain properdin, failed to inhibit tumor growth *in vivo*. It is possible that the components of NHS described herein may, however, be similar or related to the cytotoxins of Bolande and co-workers(4,5).

In vivo tumor inhibitory activity was also shown to be present in schizophrenic serum (SS). It is at present unresolved whether the activity of SS is due to the same component or components of NHS, or if SS contains additional *in vivo* tumor inhibitory factors. Federoff(3) using mouse strain A cells in tissue culture found that both NHS and SS were toxic to these cells. However, degree of toxicity was found to be greater for blood sera of schizophrenics than for sera of healthy individuals.

Summary. Normal human serum contains a heat-labile, non-dialyzable, ammonium-sulfate precipitable euglobulin fraction that inhibited growth of Sarcoma 180 cells *in vivo*. This component was found to be active between pH 5-9 and required no divalent cations. Schizophrenic serum was also found to be inhibitory to Sarcoma 180 cells. Sera of individuals with blood dyscrasias failed to protect mice against tumor development.

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Insulin Potentiating Action of Tolbutamide in Eviscerated Cats. (26080)

JOOP MADSEN (Introduced by G. Asboe-Hansen)
Institute of Medical Physiology, University of Copenhagen, Denmark

An increase in glucose uptake of hepatectomized rats, rabbits and dogs has been produced by injection of tolbutamide(1,8,9). The author has shown the same in hepatectomized cats(5). He explained this effect as probably due to a "potentiation" of insulin secreted in the animals. This explanation is supported by the present experiments.

Methods. Nembutal anaesthetized cats were eviscerated and nephrectomized. Glucose was then infused *i.v.* at a constant rate sufficient to keep the blood sugar level nearly constant. Groups of 6-7 animals were treated as follows: Two groups were injected with insulin 0.01 unit/kg *i.v.* One of these received tolbutamide 100 mg/kg *i.v.* 5 minutes before insulin injection. A third group received only tolbutamide 100 mg/kg *i.v.* The fourth group served as controls, which were injected with a volume of 0.9% saline similar to that of the tolbutamide injection in the 2 preceding groups. Blood sugar level was followed at 10 to 20 minute intervals for 2 hours before and 3 hours after injections. Blood sugar determinations were made by the Somogyi-Nelson technic(7) on heparinised plasma obtained from arterial blood samples immediately after bleeding.

Results. The results are shown in Fig. 1 and 2 giving average values of the 4 groups, blood sugar concentrations being expressed as percentages of the level at zero hour. Absolute blood sugar values at this time varied between 82 and 160 mg %.

Fig. 1 demonstrates how the hypoglycemic effect of insulin (0.01 unit/kg) is reinforced by tolbutamide. T-tests shows that corresponding points in the 2 curves differ significantly 40 minutes after the medication ($P<0.05$). After 60 minutes P was less than 0.01. It kept this value during the rest of the experiment except 75 and 120 minutes after the injections, where $0.01<P<0.05$.

Fig. 2 shows a slight effect of tolbutamide given without insulin to eviscerated, nephrectomized cats. The difference between the curves is not statistically significant at the 5% probability level. The blood sugar rises slightly in the last half of the experimental period when only saline is injected, while it remains practically unaltered during the 5 hours of the experiments when tolbutamide is given.

Comparing corresponding differences in the pair of curves in Fig. 1 and 2 it is found that the hypoglycemic effect of tolbutamide is 2½-6 times as pronounced when given with insulin as when given alone. This clearly indicates an insulin "potentiating" effect, although the difference between the tolbutamide effects in Fig. 1 and 2 can not be proved statistically at the 5% probability level with the present number of experiments. The insulin potentiation appears partly as a more pronounced, partly as a more sustained hypoglycemia than when insulin is given alone.

Discussion. The mechanism of this insulin potentiation is obscure, but the maintained hypoglycemia could be explained by an inhibited insulin degradation, as proposed by Mirsky(6). If this is the case, the feeble effect seen in eviscerated animals not given insulin (Fig. 2) might be due to preservation of small amounts of insulin remaining in the animals from before the evisceration. This possibility is at present under investigation