to the predicted value of ⅔ and hence seems to bear out the theory.

In making the plot, only the totals for the body-weight classes up to 198 lb were used because the athletes in these classes are usually trained down to the listed body weight and hence carry very little excess weight. It is interesting to note that the present world-record heavyweight total of about 1130 lb should be within the ability of a lifter of 232-lb body-weight, whereas the present record holder, Anderson, weighs about 350 lb. The existence of the linear relationship also suggests that it might be desirable to break down the present heavyweight class into at least two classes to prevent lifters who weigh over 300 lb from competing with those in, for example, the 220- to 250-lb range.

It should be emphasized that the totals plotted are in no sense "ultimates" but will continue slowly to be improved. However, the slope of the line drawn through the points plotted on a log-log basis should continue to be approximately ⅔. Also, the log-log plot can be used to determine the best weight lifter at any time, since his total will fall the farthest above the line drawn by the method of least squares through all the records. At the present time, the total for the 148-lb body class falls the farthest above the line. Hence the Soviet athlete, Kostilev, who holds the record, appears to be at present the world's best weight lifter.

M. H. Lietzke
Oak Ridge National Laboratory, Oak Ridge, Tennessee

Reference

1. T. F. Young, private communication.

28 May 1956

Surface Studies Using Ion-Exchange Autochromatography

The heterogeneous exchange between the ions on the surface of an ionic crystal and the ions in a solution has been widely used as the basis for a radioactive method for determining the "active" surface area of finely divided solids (for recent summaries, see Paneth, *1*, and Wahl and Bonner, *2*). Tracers isotopic with either the cation or the anion of the crystals may be used and should, on the basis of simple theory, yield the same calculated surface area. This is, indeed, the case in experiments in which labeled, saturated $PbSO_4$ solutions are shaken with $PbSO_4$ precipitates, but is not the case when saturated solutions containing $Sr^{90}S^{35}O_4$ are shaken with $SrSO_4$ precipitates (*3*). For the latter, the apparent areas, as determined using Sr^{90} ion and $S^{35}O_4^{--}$, are in the ratio of about 2/1. This difference is somewhat unexpected

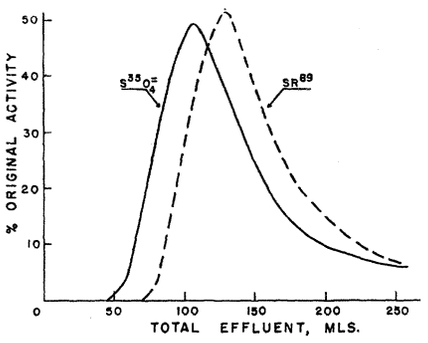

Fig. 1. Elution curve for Sr^{++} and SO_4^{--} using a $SrSO_4$ column at 25°C.

and seems large enough to warrant further study.

For this purpose a new method has been developed, the method of *ion-exchange autochromatography*. In a typical experiment, a Pyrex glass column of 1.2-cm inside diameter and 30 cm in length, contained 17.9 g of $SrSO_4$ precipitate in a length of 11 cm. The free volume was 8.75 ml. Fifty milliliters of $SrSO_4$ solution, saturated at 25°C and labeled with both Sr^{89} ion and $S^{35}O_4^{--}$ was added to the column. The column was then eluted with approximately 200 ml of saturated $SrSO_4$, and 50 successive 5-ml fractions of the effluent were collected. Suitable rates were obtained by applying suction to the bottom of the column. The column was jacketed and maintained at constant temperature.

The relative radioactivity of each sample was measured for both Sr^{89} ion and $S^{35}O_4^{--}$. Figure 1 is a typical elution curve obtained in this work for 25°C. It will be noted that the Sr^{++} and SO_4^{--} curves are separated and that the SO_4^{--} comes out ahead of the Sr^{++}. If a sufficiently long column were used, the two curves could be sufficiently displaced to make possible a reasonably complete separation of the Sr^{89} and $S^{35}O_4^{--}$ activities. Thus, when the chromatographic-column technique is applied, small differences in surface behavior can be accentuated (as compared with a single-batch experiment) and studied in detail. Further experiments varying the rate of flow of fluid, column length, temperature, and so on are in progress, and it is thought that the application of this sensitive new method will throw light on surface-exchange phenomena that are little understood at present.

By using two separately measurable isotopes, isotopic effects in surface exchange could also be studied and might be expected to be of considerable theoretical (and possibly practical) importance.

Ruth E. Krehbiel
J. W. T. Spinks
Department of Chemistry, University of Saskatchwan, Saskatoon, Canada

References

1. F. A. Paneth, *J. chim. phys.* 45, 205 (1948).
2. A. L. Wahl and N. A. Bonner, *Radioactivity Applied to Chemistry* (Wiley, New York, 1951).
3. R. M. Singleton and J. W. T. Spinks, *Can. J. Research B* 27, 238 (1949); R. M. Stow and J. W. T. Spinks, *Can. J. Chem.* 33, 938 (1955).

25 June 1956

Influence of Invasiveness, Hormones, and Amphenone on Steroids in Adrenal Carcinoma

The study reported here (*1*) dealt with serial quantitative isolation of individual steroid hormone metabolites during the localized and metastatic phases of a functional adrenocortical carcinoma. It has been established that steroid production by the metastases is unequivocally increased by adrenocorticotrophic hormone (ACTH), probably decreased by exogenous cortisone, and markedly diminished by Amphenone [1,2-bis(*p*-aminophenyl) 2-methyl propanone-1 dihydrochloride] (*2*). This response is in contrast to the relative independence from extrinsic influence generally assumed to be characteristic of this form of malignancy.

In 1951, a 42-year-old woman was observed at Montefiore Hospital, with findings suggestive of adrenal hyperactivity, including amenorrhea, hirsutism, and hypertension. Isolation of urinary steroids (*3*) revealed (Fig. 1) normal levels of dehydroisoandrosterone (D) and androsterone (A), but etiocholanolone (E) and the three major 11-oxygenated steroids (11 = OE, OH-E, and OH-A) were greatly elevated, demonstrating considerably increased adrenocortical hormone production. At surgical exploration, the left adrenal, containing an encapsulated tumor that weighed 140 g, was removed. The tumor showed bizarre cells and increased mitotic activity in focal areas. Postoperatively (Fig. 1), steroid isolation revealed a low level of hormone production, with the individual metabolites in the usual proportion, which was consistent with the clinical evidence of transient adrenal hypofunction.

In 1955, pronounced clinical evidence of adrenal hyperactivity emerged, and biopsy of intra-abdominal metastatic lesions showed adrenocortical carcinoma. At postmortem examination later, the right adrenal gland was normal. The urinary steroids were grossly altered compared with the localized phase (Fig. 1). The 11-oxygenated steroids were elevated to approximately 10 times normal levels, and the 11-desoxysteroids had changed even more strikingly. Androsterone had increased ten fold; etiocholanolone was approximately 30 times the normal level and, most striking of all, dehydroisoandrosterone (87 mg/day) had

now become the major urinary steroid.

Immediately following this control study, 20 units of ACTH per day were administered intraveneously for 3 days, and there was an immediate and impressive increase in steroid production (Fig. 2). It is emphasized that the prompt onset and magnitude of this response preclude the possibility that the contralateral adrenal was responsible for the increased steroid production. In extended experience in these laboratories, a response of the α-hydrotysteroids of such magnitude has been observed only after prolonged treatment with ACTH in normal subjects with both adrenals intact. Perhaps the most convincing evidence that the metastases responded to ACTH was the 50-mg increment in dehydroisoandrosterone, an increase never observed under any circumstances in a subject with normal adrenal glands.

Six weeks following the ACTH period and 5 weeks after biopsy, a period on cortisone administration (62 mg/day for 8 days) was studied (Fig. 2). The individual steroids were lower than any of the control periods in 1955, suggesting depression of tumor steroidogenesis. Since it was not possible to obtain a control study immediately before administration of cortisone, this conclusion is tentative.

Subsequently, the patient received treatment with Amphenone (4 g/day for 10 days) between control intervals before and after the administration of the drug (*4*). Steroid production fell precipitously though somewhat unequally (Fig. 2). Androsterone and etiocholanolone were decreased significantly but still were above normal. The major component, dehydroisoandrosterone, fell to a normal level, and the 11-oxygenated steroids dropped to nearly normal limits.

It should be emphasized that these values for 11-oxygenated compounds during Amphenone administration were maximal for there was some nonsteroidal chromogen in these areas of the chromatogram, and no arbitrary correction was made for this. It is our impression that true values for these compounds were very much lower than those recorded. These results clearly prove that Amphenone markedly depressed the steroid production of the tumor. That this inhibition was functional rather than lethal is evident from the prompt return of excessive steroid production after the drug was discontinued.

The major metabolites reported here accurately reflect two of the principal secretory products of normal as well as abnormal adrenals. The demonstration that this adrenocortical carcinoma retained residual capacity to respond to both humoral and drug influence bears significantly on the problem of endogenous and exogenous control of function of these tumors and lends encouragement

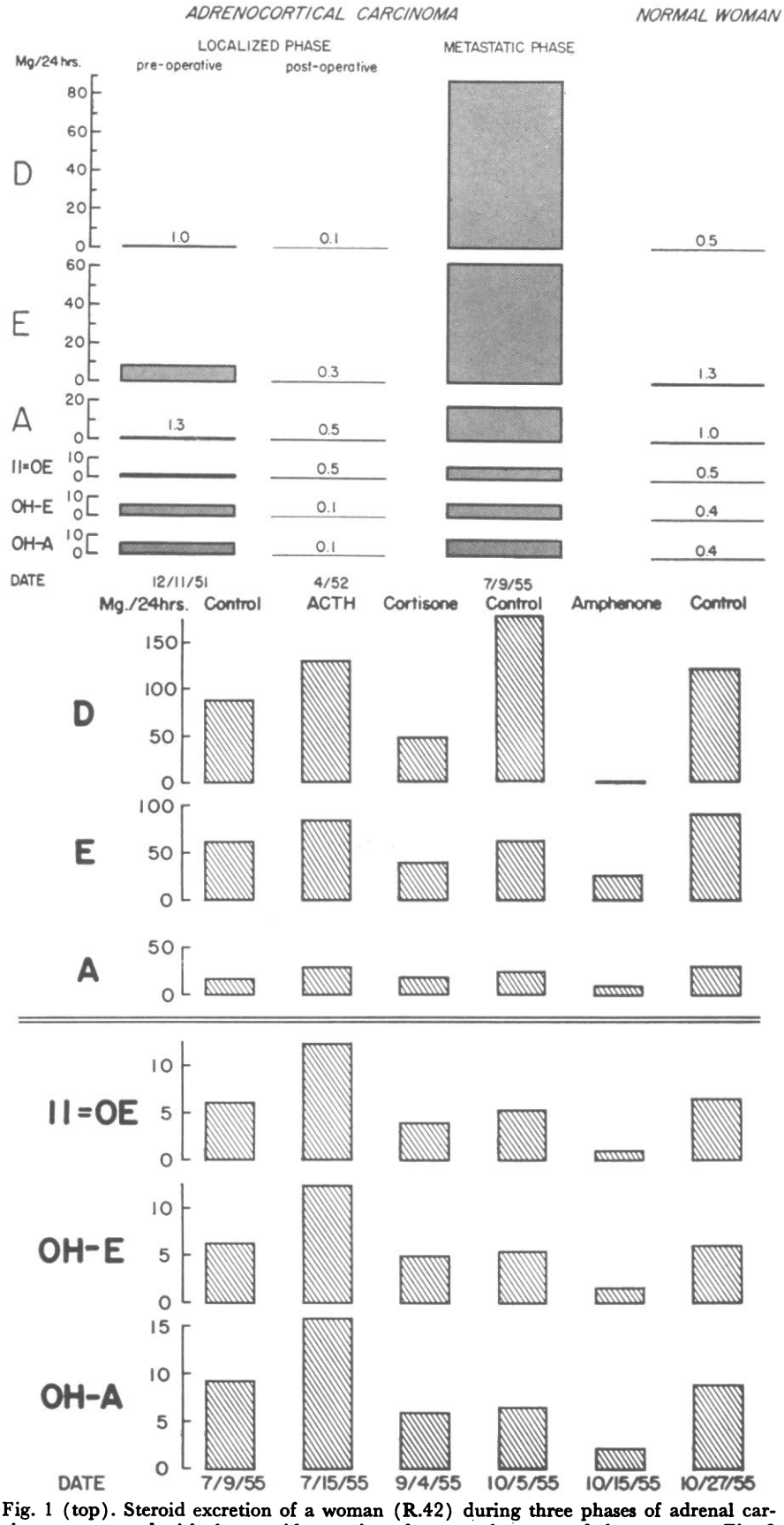

Fig. 1 (top). Steroid excretion of a woman (R.42) during three phases of adrenal carcinoma compared with the steroid excretion of a normal woman of the same age. Fig. 2 (bottom). Steroid excretion of R.42 (metastatic adrenocortical carcinoma) during control intervals and while under the influence of ACTH, cortisone, and Amphenone. D, dehydroisoandrosterone (3β-hydroxy-Δ^5-androstene-17-one); E, etiocholanolone (3α-hydroxyetiocholane-17-one); A, androsterone (3α-hydroxyandrostane-17-one); 11 = OE, 3α-hydroxyetiocholane-11,17-dione; OH-E, 3α-11β-dihydroxyetiocholane-17-one; OH-A, 3α,11β-dihydroxyandrostane-17-one.

to the search for similar effects with other neoplasms and with a broader spectrum of chemotherapeutic compounds.

T. F. GALLAGHER, ATTALLAH KAPPAS, HERTA SPENCER, DANIEL LASZLO
Sloan-Kettering Institute for Cancer Research and *Montefiore Hospital, New York*

References and Notes

1. This investigation was supported by a grant from the American Cancer Society and by research grants (C-322 and C-1540) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Md.
2. R. Hertz, *et al.*, *Recent Progr. Hormone Research* 11, 119 (1955).
3. The methods employed have been described by A. Kappas and T. F. Gallagher, *J. Clin. Invest.* 34, 1566 (1955), and earlier publications.
4. Supplied through the generosity of Roy Hertz, National Institutes of Health, to whom we express our thanks for his cooperation.

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Partial Inactivation of Lipoprotein Lipase by Bacterial "Heparinase"

Several independent lines of evidence have indicated the existence of a rather close relationship between lipoprotein lipase and heparin. Indeed, the discovery of the enzyme derived from the fact that, upon the injection of heparin, the lipase appears in the blood, where its presence is visibly demonstrable in lipemic animals (*1*). The direct isolation of lipoprotein lipase from tissues (heart and adipose) of normal animals has been reported previously (*2*). In such preparations, it is possible to demonstrate an activation of the enzyme by added heparin. Lipoprotein lipase is inhibited by protamine. It can also be inhibited by pyrophosphate, and this inhibition, at least in the preparations from rat heart, can be reversed by heparin. More recently, Robinson (*3*) has reported that the heat stability of lipoprotein lipase of postheparin plasma is decreased by passing the plasma over an anion exchange resin; the addition of free heparin to such a preparation restabilizes the enzyme.

The isolation in this laboratory of a *Flavobacterium* (*4*) that is able to use heparin as its sole source of carbon, nitrogen, and sulfur, and the preparation of bacterial extracts (*5*) that possess "heparinase" (*6*) activity have provided another tool for the study of the interrelationship between heparin and lipoprotein lipase.

The data summarized in Table 1 show the results obtained on the incubation of lipoprotein lipase with the bacterial extract. Acetone powders of adapted bacteria were extracted with water and dialyzed against 0.005*M* ethylenediaminetetraacetate and then against water overnight. The precipitated protein was dissolved in 0.025*M* glycylglycine buffer at *p*H 7.5 and diluted to a protein concentration of 1 mg/ml. The lipoprotein lipase was a purified preparation from chicken adipose tissue (*7*) dissolved in 0.025*M* NH_3 to a protein concentration of 5 mg/ml. The bacterial extract and lipoprotein lipase (0.2 ml) were preincubated together in a total volume of 0.4 ml of 0.025*M* glycylglycine which contained 0.005*M* $MgCl_2$ for 15 minutes at 24°C. In each instance, the proper control, without the bacterial extract, was run. The vessels were then heated at 40°C for 5 minutes to inactivate selectively the heparinase. Four milliliters of 10-percent albumin at *p*H 8.5, 0.05 ml of 1*M* ammonium sulfate, and 0.05 ml of 2-percent activated coconut oil (*8*) were added to all the vessels, which were then incubated at 37°C for 1 hour for the determination of lipoprotein lipase activity (*2*). The glycerol that was produced was oxidized by periodate to formaldehyde, which was then determined colorimetrically with chromotropic acid.

Under optimum conditions, the lipase can be 60 percent inactivated. The component of the bacterial extract responsible for this inactivation is a nondialyzable, heat labile substance that exhibits all of the known properties of the heparinase (*5*). These are (i) complete inactivation when heated for 5 minutes at 40°C, (ii) a requirement for one of several ions for activity (magnesium, ammonium, phosphate, arsenate, or citrate), and (iii) inactivation when incubated in the presence of 0.2*M* NaCl or phosphate buffer. The bacterial extracts have some slight proteolytic activity, but this shows none of the afore-mentioned characteristics. Also, extracts of acetone powders of bacteria that had not been adapted to heparin do not inactivate lipoprotein lipase even though they possess equal proteolytic activity. On the basis of this evidence, it is concluded that the heparinase is responsible for the inactivation of the lipase and that lipoprotein lipase is, most probably, a mucoprotein which contains a mucopolysaccharide very similar to heparin as an integral part of the molecule.

Table 1. Inactivation of lipoprotein lipase by bacterial heparinase.

Additions during preincubation	Glycerol production (D_{570})
None	0.215
Bacterial extract, 0.01 ml	0.120
Bacterial extract, 0.10 ml	0.080
Bacterial extract, 0.01 ml + 0.2*M* NaCl	0.220
Heated* bacterial extract, 0.01 ml	0.210
Bacterial extract, 0.10 ml; no Mg^{++}	0.155

* Heated at 40°C for 5 minutes.

EDWARD D. KORN
Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland

References and Notes

1. P. F. Hahn, *Science* 98, 19 (1943).
2. E. D. Korn, *J. Biol. Chem.* 215, 1, 15 (1955); E. D. Korn and T. W. Quigley, *Biochim. et Biophys. Acta* 18, 143 (1955).
3. D. S. Robinson, *Biochem J.* (*London*) 62, 18 (1956).
4. A. N. Payza and E. D. Korn, *Nature* 174, 88 (1956).
5. E. D. Korn and A. N. Payza, *Biochim. et Biophys. Acta* 20, 596 (1956).
6. The term *heparinase* is used to refer to a group of at least three enzymes (*5*) that catalyze an extensive degradation of heparin.
7. E. D. Korn and T. W. Quigley, unpublished data.
8. Activated coconut oil is prepared by incubating a commercial coconut-oil emulsion with the α-lipoprotein fraction of normal plasma. The oil is then washed repeatedly by centrifugation through 0.15*M* NaCl. This washed, activated coconut oil contains a small amount of protein, cholesterol, and phospholipid, and it is identical to chylomicrons with respect to the action of lipoprotein lipase.

29 June 1956

Man is to himself the most wonderful object in nature; for he cannot conceive what the body is, still less what the mind is, and least of all how a body should be united to a mind. This is the consummation of his difficulties, and yet it is his very being.—B. PASCAL, *Pensées.*