why only about 20% inhibition of serum oxidase activity could be obtained with EDTA. Presence of 2 ceruloplasmins has been demonstrated in serum(4) and it may be that one is much more resistant to EDTA than the other.

Summary. A method for determination of ceruloplasmin in serum is presented in which rate of oxidation of p-phenylenediamine is measured spectrophotometrically or photometrically. Correction for catalytic oxidation by free metals present is made by reading the test against a serum blank in which enzymatic activity is inhibited by azide. The effects of pH and temperature on the reaction have been investigated and normal values obtained by our method. Ethylenediaminetetraacetate significantly inhibits enzymatic oxidase activity of serum.

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Uptake of Glycine-1-C^{14} by Connective Tissue. III. Effects of Adrenal Hormones and Aging.* (25929)

HANS KOBLET AND EDWARD H. FRIEDEN (Introduced by P. Bernfeld)
Arthur G. Rotch Lab., Boston Dispensary, Boston, Mass.

Changes in connective tissue constituents in response to administration of adrenal cortical hormones have been described many times. Histological studies have afforded evidence for their involvement in metabolism of skin connective tissue and granulation tissue of a number of species of animals(1,2,3,4). Metabolic and chemical studies, including those employing tracers, support the conclusion that glucocorticoids inhibit formation of connective tissue components, especially those of the ground substance(5,6,7). However, the extent to which these hormones influence metabolism of the collagenous components of connective tissue is uncertain. Perrone(8) was unable to demonstrate an effect of cortisone upon incorporation of labeled glycine into the collagen of rat skin. Robertson and Sanborn(9), however, found that cortisone reduced (and adrenalectomy increased) collagen concentration in carrageenin granulomas in guinea pigs. Like the carrageenin granu-

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loma method, the sponge-biopsy technic introduced by Boucek(10) offers a means of studying connective tissue metabolism in a relatively uncomplicated system. The effects of adrenal cortical hormones upon incorporation of labeled glycine into sponge biopsy proteins are here described.

Methods. Female Sprague-Dawley rats were used. Unless otherwise stated, the animals were 4 months old and weighed about 200 g at beginning of experiment. The technic of sponge implantation was essentially that described by Boucek: Washed Ivalon sponges, measuring 2 x 2 x 1 cm, were dried at 100° for 4 hours, weighed, and moistened with sterile saline solution immediately before use. Two sponges were implanted under the skin of the back of each animal. In some animals adrenals were removed by the lumbar approach immediately before implanting the sponge. All animals received a stock diet and water *ad libitum*. Adrenalectomized animals received, in addition, 1.5% sodium chloride solution. Treated animals received hydrocortisone (0.1 ml daily of a suspension containing 10 mg/ml in sesame oil) beginning on 10th day after implantation of the sponge. On the fourteenth day,† 4 μC of labeled glycine‡ in 0.4 ml distilled water was injected intraperitoneally. The first sponge was removed after 6 hours; 18 hours later, the animals were killed and the second sponge, along with small samples of kidney, liver, spleen, and skin, was removed and weighed; after mincing or homogenizing, duplicate samples were fractionated into water-soluble and alkali-soluble fractions as previously described(11). Proteins in each fraction were precipitated 3 times by addition of TCA to 5%, washed twice with acetone, once each with ethanol: chloroform:ether (2:1:1) and benzene, and finally plated from benzene on tared aluminum planchets. The alkali-insoluble residue from each tissue sample was suspended in 5 ml water and autoclaved overnight at 15 lb pressure. The resulting gelatin solution was separated from any insoluble material and evaporated to a volume of 1 ml. Part of the solution was plated for counting; that the residue consisted entirely of gelatin, was confirmed by nitrogen and hydroxyproline determinations on aliquots of the solution. All samples were counted for a period long enough to assure precision of 5% or better; data are recorded as CPM/mg protein, corrected for self-absorption to a sample density of 5 mg/cm^2.

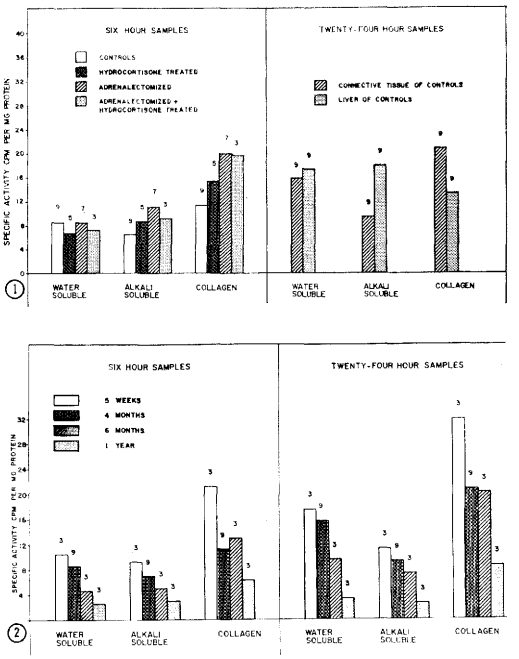

FIG. 1. (Left) Specific activities of protein fractions obtained from sponges removed 6 hr after inj. of labeled glycine (4 μC) into control and treated animals. (Right) Specific activities of protein fractions from sponge connective tissue and liver samples removed 24 hr after inj. of labeled glycine into untreated animals. Each bar represents avg for No. of animals indicated above it.

FIG. 2. Specific activities of protein fractions obtained from sponges removed 6 hr (left) and 24 hr (right) after inj. of labeled glycine (4 μC/200 g body wt) into rats of various ages. Each bar is avg for No. of animals indicated above it.

Results. The extent of incorporation of labeled glycine during 6 hours into proteins of sponge-biopsy connective tissue is shown in the left half of Fig. 1 for 4 groups of animals (untreated controls, hydrocortisone-treated non-adrenalectomized animals, untreated adrenalectomized animals, and adrenalecto-

† Preliminary studies indicated that adequate infiltration of sponge by connective tissue occurred after 2 weeks.

‡ Glycine-1-C^{14}, specific activity 3.4 mC/mM obtained from New England Nuclear Corp.

mized animals which had received hydrocortisone). In the right half of Fig. 1, data after 24 hours for sponge proteins of untreated animals are compared with those for liver after the same period of time. It is apparent that uptake of glycine by connective tissue proteins of the sponge, including collagen, is quite rapid; specific activities, after 24 hours, approximate those for liver protein.

Neither adrenalectomy, nor administration of hydrocortisone, nor a combination of the 2 procedures was found to affect significantly incorporation of glycine into water-soluble sponge proteins during 6 hours (Fig. 1, left). Uptake of glycine by alkali-soluble sponge protein during 6 hours was significantly ($p = 0.01$) enhanced in adrenalectomized animals, but in neither of the other groups. Although specific activities of collagen fractions obtained from adrenalectomized (or adrenalectomized + hydrocortisone-treated) animals were considerably higher than from controls, the variations encountered make the differences of doubtful significance ($p = 0.04 - 0.2$).

Data for sponges removed after 24 hours showed no effect of adrenalectomy or hydrocortisone treatment, whether comparisons were drawn between specific activities of the various samples ($p = 0.1$ or larger) or between ratios of activity of sponge protein to that of the corresponding liver fraction.

Neuberger and Perrone(12) found that labeled glycine was incorporated much more rapidly into tendon collagen of young rats than into that of older animals. It seemed of interest, therefore, to study the effect of age of the animal upon incorporation into sponge proteins. Accordingly, incorporation of glycine-1-C^{14} into sponge proteins of rats aged 5 weeks, 6 months, and 1 year was determined. Experimental details were as in previous experiments, except that amount of glycine injected was adjusted to 4.0 μC/200 g body weight. Data for all 4 groups are included in Fig. 2.

Discussion. Our data offer no evidence for a significant inhibitory effect of hydrocortisone upon incorporation of labeled glycine into collagen of sponge biopsy connective tissue and thus are consistent with Perrone's data for skin(8). The discrepancy between our data and those of Robertson and Sanborn (9) may be more apparent than real, since an effect sufficient to reduce collagen content of the granuloma by 20% over a period of 14 days might easily escape detection in our experiments. It may be concluded that effects of adrenal glucocorticoids upon connective tissues are mediated principally through effects upon ground substance. Thus, the observation by Sobel *et al.*(7) that cortisone administration significantly reduced hexoseamine: collagen ratios in skin and other tissues of rats may be explained by a reduction in hexoseamine-containing mucopolysaccharides, without a concomitant change in collagen content.

The most interesting finding in these experiments relates to the effect of host age upon glycine incorporation into sponge proteins. There appears to be a steady decrease in rate at which glycine is incorporated from 1 month to 1 year of age (Fig. 2). This effect is not confined to collagen, but involves the water and alkali-soluble fractions as well. This fact, as well as the fact that all sponges had been in place for the same length of time before administering labeled glycine, indicates that the results cannot be due to dilution by metabolically inert collagen present in larger amounts in sponges taken from older animals.

Several possible explanations might be advanced to explain the observation of lowered glycine uptake by sponge proteins in older animals. Of these, the most likely would appear to be (a) lowered metabolic activity by connective tissue-forming cells of the host, or (b) diminished availability of substrate as a result, for example, of decreased fluid exchange between sponge and peripheral circulation. A decision between these alternatives might be made on the basis of *in vitro* studies of rate of amino acid incorporation into sponge-biopsy slices from animals of various ages.

Summary. When glycine-1-C^{14} is injected into rats bearing Ivalon sponges implanted 2 weeks previously, significant radioactivity is found in the various protein fractions (including collagen) of the sponge connective tissue

after 6 hours. Adrenalectomy at the time of implantation exerted some influence upon the extent of incorporation into some of the fractions, but administration of hydrocortisone for 2 days to either intact or adrenalectomized animals had no effect. When studies of glycine uptake by sponge-biopsy connective tissue proteins were made in rats of various ages, a steady decrease in rate of incorporation into all fractions was observed as age of the host increased from one month to one year.

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Failure of Hypophysectomy, Adrenalectomy or Thyroidectomy to Affect Response of Non-Esterified Fatty Acids to Fasting. (25930)

H. P. BARROS BARRETO AND LILLIAN RECANT (Introduced by A. B. Eisenstein)
Dept. of Preventive Medicine, Washington University School of Medicine, St. Louis, Mo.

Recent investigations focused attention upon the role of non-esterified fatty acids (NEFA) in energy metabolism. As a consequence of rapid turnover of this fat fraction, a flexible source of energy is made available to the organism(1). The plasma level of NEFA appears to be the resultant of rates of release from adipose tissue and rates of subsequent transport and utilization. The mechanisms which control NEFA release are imperfectly understood, though hormonal control and dietary factors have been implicated. Insulin inhibits(2) while epinephrine(3), growth hormone (G.H.)(4), and adrenocorticotropic hormone (ACTH)(5) accelerate release of NEFA from adipose tissue. All these substances are active *in vitro* when incubated with epididymal fat pads. Except for ACTH, they have also been demonstrated active in the intact animal. Dietary factors also influence plasma NEFA levels. Prolonged fasting results in increased levels which are decreased again following carbohydrate meal(6,2). In addition, increased *in vitro* release noted in adipose tissue obtained from fasting rats is decreased by adrenalectomy or hypophysectomy(7,4). These observations suggested that response of NEFA to diet is dependent upon hormonal control. The present study was undertaken to delineate the role of certain hormonal factors on *in vivo* response of plasma NEFA to fasting.

Materials and methods. Sprague-Dawley rats weighing 150-200 g were used. Hypophysectomized rats were obtained from Hormone Assay Lab. Purina chow diet and bread supplements were provided. Studies were performed approximately 2 weeks following surgery. Adrenalectomy and thyroidectomy were performed under ether anesthesia. Sham operation, simulating adrenalectomy was also performed. Animals were studied 5-8 days after operation. Adrenalectomized rats received 1% saline in place of drinking water. All animals, unless otherwise stated, were fed standard Purina chow and provided water *ad*