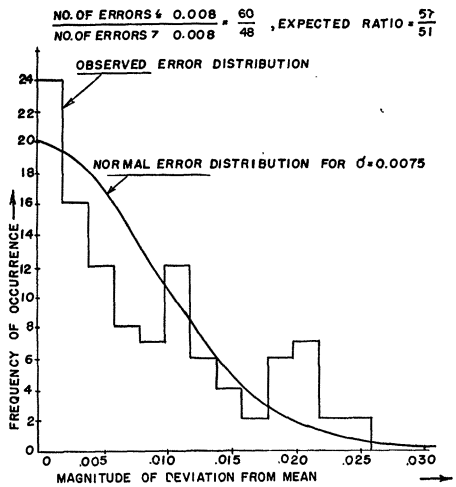

FIG. 6. Statistical summary of data.

termine its usefulness. A radium source was used and the counting rate, i.e., the efficiency in arbitrary units, was measured at the center of the counter circle and at several values of θ for each of two values of x. The results are shown plotted in Fig. 5, with arbitrary units for the ε scale. The bold circle drawn in indicates the value of ε_0 at the center of the counter circle, and it is seen that no value of ε differs from this center value by as much as 5%, indicating the validity of the analysis. Also, the average value of ε over all the measurements taken at the greatest value of x (0.2) is less than 2% greater than the center value, and the average value of ε over the smaller value of x (0.1) is practically the same as the center value, i.e., within the probable statistical error of the measurements. These averages are more significantly related to the measurements of distributed sources than are the single measurements made at particular values of θ. That these averages are appreciably smaller than the 5% that might have been expected is probably due to the variation of ε with α. This, by assumption 3 above, is assumed to be insignificant. At large values of x this variation is further increased by the thick lead shield whose slit exposes the counter to the source in a variable manner as x varies.

The probable statistical error of the measurements is considerably smaller than the observed variations. The asymmetry observed in Fig. 5 is almost certainly owing to the fact that one of the counters in this run of measurements does not have the same characteristic as the other three and therefore is counting at a higher rate. Fig. 6 shows the summary of a statistical analysis of the data, and it can be seen that the distribution of the statistical deviations of the measurements is in reasonable agreement with a normal error curve, as taken from the *Handbook of Chemistry and Physics* (30th Edition, pages 203–208).

It is expected that with this apparatus measurements of radiation dosage can be made to an accuracy approaching a few percent, subject to variations of absorption that may occur from one (human) sample to the next. We are attempting to determine the uncertainty to be expected from the greater absorption presented by the patient's neck to the counters behind the neck. Since the body is mostly matter of low atomic number, we expect the absorption will be low and very probably negligible for clinical purposes. We are also investigating the magnitude of the variation of ε with height of source above or below the plane of the four counters.

In a subsequent communication we plan to report upon this latter aspect and upon the clinical appraisals of the apparatus herein described.

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Isolation of a Steroid Hormone from the Adrenal-Vein Blood of Dogs[1]

Don H. Nelson, Hans Reich, and Leo T. Samuels

Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City

Lipid soluble material which protects adrenalectomized animals against cold has been demonstrated by Vogt (*5*) in blood obtained by cannulation of the adrenal vein of dogs. From the same source Paschkis *et al.* (*3*) have also found material causing liver glycogen deposition. As is well known, various active steroids have been isolated from adrenal tissue (*4*). According to the recent work of Haines *et al.* (*1*), the most abundant of these in fresh adrenal tissue is 17-hydroxycorticosterone (Kendall's compound F). The actual compound or compounds excreted by the adrenal cortex when stimulated by the pituitary adrenocorticotropic hormone have not, however, been identified. The development of a quantitative technique for estimation of cortical steroids in adrenal-vein blood (*6*) enabled us to isolate a steroid from this source.

The left adrenal vein of heparinized dogs, anesthetized with nembutal, was cannulated. The dogs were then injected with Armour's ACTH[2] and blood was collected at intervals for 2–4 hr. In some cases a second injection of ACTH was then given and the collection of blood continued as before.

The blood was diluted with an equal volume of water and extracted four times with an equal volume of ethyl ether or chloroform. These extracts were evaporated to dryness, taken up in 70% ethanol, and extracted three times with hexane. The ethanol fraction was dried, dissolved in chloroform, and chromatographed on a magnesium silicate-celite column, using progressively increasing concentrations of ethanol in chloroform. A small

[1] This study was supported by grants from the National Cancer Institute, U. S. Public Health Service; the American Cancer Society; and Ciba Pharmaceutical Products, Inc.

[2] The ACTH was supplied through the courtesy of Dr. Edwin E. Hays and Dr. F. R. Mote of Armour and Company.

amount of material having an absorption band at 240 mμ wavelength was found in some 2% ethanol fractions. This absorption band is characteristic of steroids containing the α–ß unsaturated ketone structure. Definitely separated from this was a much larger amount of material giving this absorption which appeared in the late 4% and early 6% ethanol fractions. On oxidation with periodic acid this material yielded formaldehyde (*2*).

The last two fractions mentioned were accumulated from several dogs. The crude material was acetylated and sublimed in high vacuum at 180° C. Most of the material sublimed. The sublimate was chromatographed on aluminum oxide. Material giving the 240 mμ absorption appeared in the benzene-ether fractions. On crystallization from ether pentane, the mp of the compound was 212°–220° C (corrected).[3] A sample of compound F acetate obtained through the courtesy of Dr. Harold Mason of the Mayo Clinic gave 214°–220.5° C mp (corrected). A mixture of the two compounds gave the same value, 214°–220° C. On the other hand, a mixture of the unknown with a known sample of cortisone acetate (mp 227°–236° C) gave a significant depression (mp 197°–209° C).

Chloroform solutions of both the unknown acetate and the sample from Dr. Mason gave infrared spectra indicating an α–ß unsaturated ketone, a keto group on carbons 11 and/or 20, an alcohol group, and an acetoxy group. The absorption of chloroform in the fingerprint region was so great, however, that positive identification from this region could not be made. The compound was too insoluble in carbon disulfide to give a spectrum. Attempts to obtain a satisfactory dry film with the small amount of material which remained were unsuccessful and must await accumulation of more substance.

When treated with chromic acid the acetate underwent oxidation and a compound was isolated which became opaque at 70°–100° C and melted at 220°–229° C (corrected). The small amount of material did not permit further purification. A known sample of compound E acetate showed opacity in the same range and melted at 227°–236° C (corrected).

The solubilities and region of elution from both magnesium silicate and aluminum hydroxide indicate a highly oxygenated polar steroid. The ultraviolet absorption spectrum and the formation of formaldehyde on oxidation with periodic acid place the compound among those having an α–ß unsaturated ketonic group in ring A, and a ketol side chain similar to the active adrenal steroids. The infrared spectrum confirms the presence of these groups and indicates at least two alcohol groups, one of which was easily acetylated. The mp of the acetate is similar to that of 17-hydroxycorticosterone acetate, is not depressed when the acetate is mixed with a known sample of this compound, and rises on oxidation with chromic acid toward that corresponding to cortisone acetate. The compound isolated from the blood flowing from the adrenal veins of dogs, therefore, appears to be 17-hydroxycorticosterone.

[3] Melting points were determined on the Kofler micro hot stage and measured from first softening to complete liquefaction.

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The Effect of 2,4-Dichlorophenoxy Acetic Acid and Various Other Substances upon the Respiration of Blue Lupine Seedling Roots

Fred R. West, Jr.[1] and James H. M. Henderson[2]

The G. W. Carver Foundation, Tuskegee Institute, Alabama

The outstanding capacity of 2,4-D, the synthetic plant hormone, to control certain phases of plant growth has been known for a number of years (*8*). Concurrent with growth and exerting some regulating influence upon it is the process of respiration. In this study, the process of respiration as affected by a synthetic hormone, 2,4-D, is considered from the over-all viewpoint of oxygen uptake and carbon dioxide release, as shown in the respiration of excised seedling roots.

Materials and methods. Selected seeds of *Lupinus angustifolius* L., first treated with a wetting agent (Teel), then sterilized in a 0.25% formaldehyde solution, were germinated in sterile Petri dishes on moist filter paper at a temperature of 22° C. About three days were required for germination of roots of desirable size, namely, 3 cm, giving a dry weight of approximately 5 mg. Two to three were used per flask, suspended in 0.1 M KH_2PO_4. The experimental procedure was that of the conventional Direct Method of Warburg.

Experimental results. The effect of 2,4-D upon the respiratory rates of blue lupine. In setting up this experiment, it was assumed that 2,4-D exerts a specific inhibitory effect upon plant cells. The concentration of 2,4-D necessary to bring about an inhibition of oxygen uptake was determined over a range from 0.05 to 0.00001 M. A concentration of 0.05 M is inhibitory at both pH 4.5 and 5.0 (29% and 27% respectively), whereas at 0.01 M the inhibition is slow and gradual, although obvious (20% and 17%). Below 0.001 M (15%) significant inhibitory effect was absent. Auxin (IAA) at a concentration of 0.0016 M showed neither an inhibition nor an acceleration at pH 5.0.

The effect of substrates at a concentration of 0.01 M upon the respiration of 2,4-D-treated and untreated roots. Various compounds and substrates associated with plant metabolism were used in an attempt to overcome inhibition by 2,4-D (0.05 M) and thus give some clue as to wherein the mechanism might be located. These sub-

[1] Present address: St. Augustine's College, Raleigh, North Carolina.

[2] Present address: Kerckhoff Biological Laboratories, California Institute of Technology, Pasadena 4.