rays) per human being and per unit time. Table 1 shows a preliminary comparison based on an estimated average body weight of 80 kg.

TABLE 1

Element	Wt. % in human body	Total element in body (grams)	Literature source	Specific act. dis./min. and 1 gram element	Nature of emitted particle	Dis./min. and man
Potassium	0.35	280	Sherman (2)	1,340	β	380,000
Carbon	18.0	14,400	"	10.5	β	150,000
Radium	$\approx 10^{-12}$	$\approx 8 \cdot 10^{-9}$	Vernadsky (3)	$2.22 \cdot 10^{12}$	α	$\approx$18,000
	$\approx 5 \cdot 10^{-12}$	$\approx 4 \cdot 10^{-9}$	Evans*	"	α	$\approx$9,000

*Private communication from Robley D. Evans, Massachusetts Institute of Technology

The intensity of cosmic radiation at sea level corresponds to approximately 1 meson per minute and per cm.[3] Therefore, depending on whether the human being is in a standing or lying position, *i.e.* on his "cross section," the number of penetrating particles ranges approximately between 1,500 and 7,500 per man and per minute.

Thus, comparing these data, we see that cosmic radiocarbon occupies second place. Based on number of disintegrations, it is a little below half of potassium, but very much above radium and other natural radioelements. Only because of the low *specific* activity of carbon has it escaped detection so far. We believe that the biological significance of the radioactivity of carbon in living matter cannot be evaluated in physical terms alone; specific biological and biochemical factors may also play a role. It will be up to future research to establish these.

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IN THE LABORATORY

Bioassay by Direct Potency Estimation[1]

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Two main approaches are used to determine the potency (Py) of a drug, *i.e.* the ratio between threshold equipotent doses of the standard (S) and the unknown drug (U) (Py = S/U). One procedure uses the median effective doses (ED_{50}) of standard and unknown as expressions for S and U, respectively, and operates by determining these two values in separate populations of test animals. Another assay method compares the standard and the unknown drug on the same test object or animal. Usually the aim is first to find in each of various test individuals a pair of equipotent doses of the standard and the unknown (s^1, u^1; s^2, u^2; s^3, u^3; etc.). each such dose level complying with a set end-point in a critical range of effect, and then to obtain from these dose pairs a number (N) of equality statements (E), each E representing an estimate of the intraindividual potency in the respective test object ($E^1 = s^1/u^1$; $E^2 = s^2/u^2$; $E^3 = s^3/u^3$; etc.). Py is then defined as the average of the intraindividual potencies $\left(\frac{\Sigma E}{N}\right)$. Thus, in the latter method, potency is evaluated directly on the basis of intraindividual potency statements and not by a preceding determination of S and U which is characteristic of the former method. The former may therefore be called an indirect, and the latter a direct, method of potency evaluation.

The indirect method does not need any special biostatistical basis. Determinations of ED_{50} have long been placed upon an elaborate mathematical basis, and their adoption has satisfied all biostatistical requirements of such a method. On the other hand, the direct method has two major shortcomings: It lacks a rigorous biostatistical means for defining the significance of the result, and its purpose is accomplished in an uneconomical trial-and-error procedure. Prior to arriving at the respective doses s and u, one has to perform tests with a varying number of doses either higher (h) or lower (l) than s or u, respectively, *i.e.* with doses h_s and l_s of the standard and doses h_u and l_u of the unknown. The data from all these preliminary tests are ultimately discarded as being "aberrant."

Only in one variant of direct procedure, the "method of approximation" (*2*), are these aberrant doses utilized for obtaining a rough estimation of the significance of the result. They are paired, both mutually and with the respective s and u values, to form additional statements pertinent to potency, namely, statements H of maximum potency (signifying Py < H), expressed by the ratios h_s/l_u, s/l_u and h_s/u, and statements L of minimum potency (Py > L), expressed by the ratios l_s/h_u, s/h_u and l_s/u. All these H and L values are then arranged in

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numerical progression; they approximate the true Py value from opposite directions, and the range of overlap indicates the deviation from the true Py. For example, the series of experimental H, L, and E data in columns 1 to 3 of Table 1 would indicate a mean Py of 27.2 and a deviation of ±16.5 (or ±45 per cent) from a midvalue of 36.5.

TABLE 1

POTENCY ESTIMATION OF THE 3-(1-METHYL-OCTYL) ANALOG OF SYNTHETIC TETRAHYDROCANNABINOL*

1	2	3	4	5	6	7	8	9	10
H	E	L	n_H	n_E	n_L	M Py of group	$\frac{n_H+n_E}{+n_L}$	λ_H	Probit
60									
60			3	0	0				
60						56.0	6	5.5/6	6.37
52	52		2	1	0				
52									
50	50								
50	50		2	2	0				
48									
48			3	0	0				
48						46.8	13	9/13	5.50
46	46								
	46		1	2	0				
	42	42							
	42		0	2	1				
40	40	40	1	1	2				
		40							
	36.4		0	1	0				
34		34	1	0	2				
		34							
33.6			1	0	0	32.9	17	9/17	5.15
33.4	33.4		1	1	0				
		33.2	0	0	1				
32	32	32	2	1	1				
32									
30.8			1	0	0				
	30	30	0	2	1				
	30								
	27	27	0	1	1				
		25.2	0	0	1	26.2	10	2/10	4.16
	24	24	0	1	1				
		23.4	0	0	1				
		21.2	0	0	1				
	20	20							
	20	20							
		20	0	2	5				
		20							
		20				18.4	11	1/11	3.51
		18	0	0	2				
		18							
		16	0	0	2				
		16							

N = 57

$2s' = 50.8 - 23.6 = 27.2$

$\sqrt{2N'} = 10.68$

Py = 35.0

s.e. = $\frac{2s'}{\sqrt{2N'}} = \pm 2.54$

* Test function: ataxia activity in calibrated dogs.
Standard: synthetic tetrahydrocannabinol.

Even in this variant, the direct bioassay procedure is mathematically unsatisfactory, and preference is given whenever possible to the indirect method. Yet, of necessity, the direct method is employed whenever the test objects (e.g. isolated organs, monkeys, dogs) are from populations with excessive interindividual variation or whenever intraindividual comparison is the procedure of choice. This signifies a wide field of actual use of, and too great a need for, procedures for direct Py estimation to dismiss them summarily as devoid of mathematical background (*1*) rather than to furnish constructive assistance for their improvement.

Obviously, conventional biostatistical methods can readily be applied to the data from such intraindividual dose comparisons. The totality of data on maximum, intraindividual, and minimum potency, collected for the above procedure of approximation, furnishes useful information, and the alignment of such data in a series of approximation (compare Table 1, columns 1 to 3) offers an appropriate starting point for a statistical likelihood solution.

The likelihood λ_H that any of the tentative potencies expressed by H or L values or both is greater than the true Py varies along the approximation series. If the total range is subdivided into appropriate groups of tentative potency, the likelihood that the mean Py of the individual group ($M\ Py = \frac{\Sigma H+\Sigma E+\Sigma L}{n_H+n_E+n_L}$) is greater than the true potency value Py is expressed by the proportion between the number of H statements (n_H) and the total number of potency statements ($n_H+n_E+n_L$) available for the group. The ambiguous E values must be assumed to express an equal likelihood that the median Py value of the group is too high and that it is too low. Accordingly, the final likelihood for a group is $\lambda_H = \frac{n_H+n_E/2}{n_H+n_E+n_L}$. The λ_H values coordinated to each M Py are calculated and converted into probits by reference to a probit table or to a table of integral of the normal probability curve. The probits, when plotted against the logarithm of their M Py, approximate a straight line. The abscissa value of the intersection of this line with the probit ordinate of 5 is an estimate of the true potency Py having a λ_H of 0.5. The standard error (s.e.) may also be determined from the same graph according to the formulation s.e. = $\frac{2s'}{\sqrt{2N'}}$, where 2s′ is the difference between the abscissa values of the intersections of the experimental line with the probit ordinates of 4 and 6, and where N′ is the number of potency statements within the range of probits 3.5 and 6.5.

The total procedure is illustrated by the example in Table 1, in which the biostatistical evaluation is shown applied, in parallel with the "method of approximation," to the same experimental data. Fisher's probit table and a graphical interpolation procedure comparable to that of Miller and Tainter (*3*) were employed after calculation of the M Py and λ_H values. The Py value obtained differs greatly from that computed by the "method of approximation," and the s.e. is much smaller than the range of deviation in the series of approximation. It should also be mentioned that the good agreement between the roughly computed midvalue of Py and the mathematically estimated Py value is merely accidental and that frequently there is considerable divergence between the results of the approximation method and those of the method herein outlined.

The suggested procedure for direct potency estimation has been found expedient in numerous bioassay problems, e.g. in

assaying spasmolytic and antihistaminic drugs in isolated strips of surviving gut; laxatives in terms of stool softening in rhesus monkeys; marihuana-active substances in terms of ataxia in dogs; and sympathomimetic agents in terms of ability to elevate the cat's blood pressure. The task of assaying biological activity by intraindividual comparison is not limited to drugs, but arises in many cases in which the potency of commensurable stimuli—agents, factors, etc.—has to be correlated numerically on the basis of a common response. It is believed that the principles of this procedure may be serviceable in various disciplines other than pharmacology.

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Identification and Chromatography of Androgens as Their 2,4-Dinitrophenylhydrazones

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Frequent use is made of 2,4-dinitrophenylhydrazine (DNPH) for the characterization of aldehydes and ketones, and the chromatographic adsorption of a number of the hydrazones of DNPH is described by Strain (*2*). However, the reagent does not appear to have been widely used in the study of steroid ketones. Veitch and Milone (*3*) have published a method for the estimation of estrone as the 2,4-dinitrophenylhydrazone, but their procedures seem not to have been applied to androgens. These studies suggested the possibility of using DNPH for the isolation and characterization of the constituents of commercial androgen preparations. In 1941 Coffman (*1*) reported the preparation of several androgen esters of p-phenylazobenzoic acid and was able to resolve mixtures of several of these colored esters chromatographically. In dealing with commercial products, however, this type of derivative could not be expected to distinguish between free and esterified androgenic steroids.

This paper constitutes a preliminary report of the preparation and properties of the DNP-hydrazones of testosterone, testosterone propionate, methyl testosterone, androsterone, and dehydroisoandrosterone. First attempts to prepare the hydrazone of one of these androgens (testosterone propionate) directly in an oil solution indicate that such a procedure is possible and may be developed into a useful analytical method. Over-all yields of about 60 per cent have been obtained to date. Details will be published as soon as the techniques are perfected.

The chromatographic separation of mixtures of some of these hydrazones has been accomplished, which indicates the possible application of the reagent to urinary extracts of ketosteroids for investigations in androgen metabolism.

[1] Thanks are due Mrs. Doris C. Chambers, of the Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, for carrying out the nitrogen analyses reported.

A 10- to 15-mg. sample of each androgen, dissolved in 1–2 cc. of ethanol, was allowed to react with a 10 per cent excess of reagent (prepared by adding 2 or 3 drops of concentrated hydrochloric acid to the calculated amount of the free base dissolved in a minimum of hot alcohol). and the mixture boiled for about 2 minutes. Frequently the hydrazone precipitated immediately from the hot solution, but if it did not, water was added to the point of cloudiness. On cooling, precipitation occurred. The precipitate was filtered off, washed with water to remove hydrochloric acid, and dried *in vacuo* over phosphorus pentoxide. After solution in benzene, it was adsorbed on activated alumina (Aluminum Ore Company, Grade F 20, minus 80 mesh), and the chromatogram developed with 10–15 per cent chloroform in benzene. On this adsorbent the bands formed may be brown, orange, or yellow, depending upon the individual compound and to some extent on the developing mixture. Following development, either the column was extruded and cut or, if extrusion proved difficult, the colored zones were dug out with a spatula. The hydrazones of the androgens prepared to date are all readily soluble in benzene and chloroform, moderately so in hot alcohol, but only slightly soluble in ligroin.

After elution with chloroform and filtration, the solvent was removed by distillation under reduced pressure and the hydrazone recrystallized from hot aqueous ethanol to constant melting point. Crystallization from benzene-ligroin pairs produced no change in melting point, but the color of the crystals was usually different from that seen when alcohol was employed. Yields of 95–100 per cent of theory are readily obtained.

TABLE 1

Androgen	M.P.*	Crystalline form and color	N found (%)	N calculated (%)
Testosterone	202.5°–203.5°	Red needles	11.62	11.96
Testosterone propionate	209.9°–210.5°	" "	10.67	10.68
Methyl testosterone	221.5°–223.5°	Orange "	11.41	11.86
Androsterone	232.5°–234°	Yellow "	11.68	11.91
Dehydroisoandrosterone	241°–242°	Yellow micro needles in thick mat	—	—

* Melting points were determined with an aluminum block apparatus calibrated by means of the U.S.P. melting-point reference standards, but are not otherwise corrected.

Table 1 lists the data for the androgen hydrazones prepared to date.

When a mixture of the hydrazones of testosterone and testosterone propionate was subjected to chromatographic adsorption, as described above, the constituents were readily separated and recovered in yields of 97 per cent and 91 per cent respectively, the fractions being identified by melting-point determination after recrystallization. The derivative of testosterone was adsorbed at the top of the column and that of the propionate below.

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