with five parts of a favorite food and offered to a cat that had fasted 24 hours. If it did not eat promptly, the food was taken away and the procedure repeated 12 or 24 hours later. Animals that would not eat at that time were not used for these experiments. It is essential that they eat all of the dose quickly (in about 5 or 10 minutes), since, if the food is eaten too slowly, the alloxan is diluted to below its cytotoxic level.

The dose varied from 0.5 to 1.0 gram/kg. and was higher for immature animals than for mature ones. Six to 12 hours after the alloxan meal, the animals were given milk, and the next day they were fed their usual diet of milk and fresh raw meat.

Results. Of the 14 cats that ate the food mixed with alloxan, two ate it too slowly, and one vomited some of the food. These three did not attain a diabetogenic concentration of alloxan and seemed unharmed by their experience. A fourth cat developed a complete anuria and died at the end of 72 hours.

The 10 cats that developed diabetes showed a marked albuminuria on the first day and had both albumin and sugar in the urine on the second day. In some animals the urine passed during the first 24 hours was of a bright red color, due to the excretion of murexide. Four of these cats showed upper respiratory irritation with sneezing and frothy mucus at the external nares. One had conjunctivitis, and one had blood in the stool on the sixth and seventh days.

The experiments were terminated to permit blood and tissue studies: 2 cats on the third day; 3 on the eighth day; and 1 each on the sixteenth, nineteenth, twenty-first, thirty-sixth, and sixty-fourth days. The average blood sugar of the five animals sacrificed on the third to the eighth day was 259 mg. per cent, and of the 5 cats sacrificed from the sixteenth to the sixty-fourth day it was 245 mg. per cent.

Specimens of the pancreas, adrenal, pituitary, liver, and kidney were taken and fixed in modified Bouin's solution. Sections were stained by the method of Gomori (*1*) and with hematoxylin and eosin.

Histopathology. In the animals sacrificed early, the pancreas showed pyknotic nuclei and fragmentation of the beta cells; later, some islets showed atrophy and hyalinization.

The adrenal cortex showed most of the injury to the cells in the fascicular layer. In cases exhibiting severe damage there were areas of focal necrosis and hyalinization. The medullary portion seemed to escape injury.

The anterior lobe of the pituitary showed damaged areas varying from hydropic degeneration with pyknotic nuclei to necrosis with hyalinization and cystic degeneration. There was no evidence of injury to the posterior lobe.

The liver exhibited changes varying from congestion of the sinusoids, with granular degeneration of the cytoplasm and chromatolysis of the nuclei, to small and large areas of necrosis and fatty degeneration, involving in some cases more than half the liver cells.

In the kidneys there was mild congestion to marked swelling of the glomerular tufts which in some cases obliterated the space between Bowman's capsule and the tuft. The epithelium of the convoluted tubules showed hydropic degeneration, necrosis, and desquamation into the lumen. The straight tubules frequently contained hyaline casts. This acute injury to the kidneys tended to recovery. No distinct kidney damage was observed in some of the animals allowed to live a longer time.

Discussion. In these feeding experiments the severity of the diabetes seemed to be modified by the damaging effect of alloxan upon the adrenal cortex and the anterior lobe of the pituitary. This has been found true in surgically induced diabetes (*3*), when the adrenals or pituitary are removed before total ablation of the pancreas, and in alloxan diabetes (*2*). It is for that reason, we think, that these animals did not need glucose to tide them over the hypoglycemic stage, or insulin for the hyperglycemia.

From the work of Tipson and Ruben (*4*) it appears probable that alloxan (or its reduction products) occurs normally in animal bodies. They obtained indications that it occurs in highest concentration in the liver, which may be the organ that changes it to a less toxic substance, e.g. urea.

When alloxan is introduced into the animal body via the alimentary canal, it reaches the liver first and in highest concentration. The ensuing destruction of liver cells results in impaired liver function, which may be the reason our animals have shown more adrenal, pituitary, and kidney damage than in experiments reported by others in which the drug was introduced parenterally.

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Sex Hormonal Action and Chemical Constitution

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The following communication presents a new hypothesis regarding the essential chemical and structural features sufficient for male and female sex hormonal activity as evidenced by comb growth in the

capon and production of estrus in the ovariectomized rat.

In 1941 Giacomello and Bianchi (*8*), through crystallographic studies, revealed that estrone and diethylstilbestrol are molecules of identical length, 8.55 A. This fact has suggested a means of classifying all substances showing estrogenic activity if the hypothesis is made that an optimum distance (D) of 8.55 A. between the hydroxyl or keto groups in the estrogens is essential for maximum activity. Such an hypothesis gives a division of the estrogenic substances into four classes:

I. Substances in which the distance, D, is very nearly the optimum, 8.55 A. These substances show the highest estrogenic activity.

II. Substances in which the distance, D, is appreciably larger than 8.55 A. These substances show decreasing estrogenic activity in proportion to the deviation from the optimum distance, 8.55 A.

III. Substances in which the distance, D, is appreciably less than 8.55 A. These substances also show decreasing estrogenic activity in proportion to the deviation from the optimum distance, 8.55 A.

IV. Substances which have the proper distance, D, but possess no hydroxyl or keto groups.

TABLE 1

Group	Substance	Distance (A.)	Activity (R.U.)
	trans-4,4′-dihydroxy-α, β-diethylstilbene (*3*)	8.55	.3 γ
	trans-1,2-di-α-(4-hydroxynaphthyl)-1,2-diethyletheylene (*2*)	8.56	<10 γ
I.	1-methyl-2-(4-hydroxyphenyl)-3,4-dihydro-6-hydroxynaphthylene (*10*)	8.56	.5 γ
	3,9-dihydroxy-5,6,11,12-tetrahydrochrysene* (*12*)	8.75	10 γ
	1,3-di-(4-hydroxyphenyl)-1,2-diethylpropane (*13*)	9.8	5 mg.
II.	1,4-di-(4-hydroxyphenyl)-2,3-diethylbutane (*1*)	12.0	inactive
	2,8-dihydroxy-5,6,11,12-tetrahydrochrysene (*4*)	9.45	150 γ
	trans-3,3′-dihydroxy-α, β-diethylstilbene (*9*)	7.7	less than 4,4′ analogue
III.	trans-2,2′-dihydroxy-α, β-diethylstilbene (*9*)	5.9	less than 3,3′ analogue
	p,p′-dihydroxydiphenylether (*6*)	8.0	100% estrus with 80 mg.
	p,p′-dihydroxydiphenyl (*6*)	7.1	100% estrus with 100 mg.
IV.	triphenylchloroethylene (*7*)	8.56	65 γ

* Number the chrysene ring as in *Chemical Abstracts*.

Examples of each of these classes are listed in Table 1 together with the calculated distances, D[1], and the biological activities of the compounds.

The high activity of the substance, triphenylchloroethylene, indicates the critical distance (D = 8.55 A.) to be the distance between two hydrogen bond-formers which may be –OH groups (keto groups reducible or enolizable to –OH) or simply hydrogen atoms that have been activated by the inductive effect. The inductive effect is enhanced by the insertion of a chlorine atom in this latter case. This hypothesis is further borne out by the following facts:

1. Hydroxy derivatives are always much more active estrogens than the corresponding keto analogues.
2. Ethers of the sex hormones decrease in activity in the order of difficulty of hydrolysis. Ethers and esters which are very stable to hydrolysis are inactive (*5*).
3. Triphenylchloroethylene is a more powerful estrogen than triphenylethylene. This may be accounted for by the fact that the parahydrogens of this latter compound become stronger hydrogen bond-formers when chlorine is substituted for the ethylenic hydrogen by the inductive effect.
4. Tetraphenylethylene and diphenylethane show no estrogenic response in 100-mg. doses in spayed rats, thus indicating the importance of the chlorine in producing a highly active compound (*6*).

Considerations similar to the above have led to an hypothesis basic to androgenic activity. All of these ideas are summarized in the following, together with certain experimental data and predictions that have been made.

A given substance may be estrogenic if it consists of a rather large, rigid, and inert molecular structure with two active hydrogen bond-forming groups (e.g. phenolic hydroxyl groups) located at an optimum distance of 8.55 A. from one another. In particular, the substance trans-p,p′dihydroxyazobenzene meets the requirements for an estrogen (*11*) and, in spite of its obvious chemical and physical differences from the natural and synthetic estrogens, shows definite estrogenic activity in dosages of from 10 to 15 mg. injected subcutaneously into spayed rats and in much smaller dosages when applied directly to the vagina.

If the active groups are at a distance of approximately 9–10 A. and are of somewhat weaker hydrogen bond-forming character (e.g. secondary alcoholic hydroxyl groups), then the substance may have androgenic activity. The prediction is made, upon the basis of this hypothesis, that certain nonsteroid substances will show male hormonal activity (e.g. the perhydro derivatives of trans-diethylstilbestrol and perhydro-2(p-hydroxyphenyl)-6-hydroxynaphthalene). It is also noted that the dosage required to produce an androgenic effect in rats by the most active androgens is vastly greater than the quantity of estrogen needed to produce estrus in the rat. This may be explained by the greater hydrogen bond-forming power of "estrogenic hydrogen" (which is usually due to phe-

[1] The distances were calculated by means of bond angle and distance values taken from L. Pauling, *Nature of the chemical bond*. Ithaca: Cornell Univ. Press, 1942.

nolic –OH groups) as compared to the weaker hydrogen bond-forming power of the androgenic hydrogen (e.g. secondary alcoholic hydrogen).

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Effect of Altitude Anoxia in Provoking Relapse in Malaria

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The opinion is widely held that anoxia tends to precipitate relapse in individuals with latent malaria (*1*). This view, founded on clinical observation over a period of many years, received additional support during World War II when, with the increase in both the incidence of malaria and air travel, relapses of malaria were observed to occur following aerial flight. Recently, Gajewski and Tatum (*2*), studying the phenomenon of relapse in avian malaria, succeeded in inducing relapse in from 2 to 7 days in all of more than 100 canaries with latent *P. cathermerium* infection by exposing the birds continuously to an oxygen tension of approximately 75 mm. Hg.

In the present study an attempt was made to induce relapse in human subjects using a short, yet moderately severe, anoxic assault such as might occur during high-altitude flight. Fifty overseas returnees from various Army Air Force installations who gave histories of two or more recent attacks of malaria were the subjects. They were exposed for 1 hour in a low-pressure chamber to a simulated altitude of 18,000 feet without supplementary oxygen (oxygen tension, approximately 80 mm. Hg.).

Since the subjects' statements were the sole source of information in obtaining data as to the number, circumstances, and dates of their previous attacks, it was not possible in all cases to separate reinfections from relapses; therefore, only the total number of previous attacks was recorded in each case. While the subjects were at the simulated altitude, continuous oximeter readings of oxyhemoglobin concentration in the blood were made. Thick blood smears were made immediately before and immediately after the chamber flight, and daily thick blood smears were made for 5 days thereafter. The subjects remained in the hospital under observation for a minimum of 6 days following the chamber flight, and at the time of hospital discharge were instructed to report subsequent relapses. All subjects, with the exception of one individual who had finished quinine treatment of his last relapse only 3 days before the chamber run, had discontinued atabrine or quinine administration 15 days or more prior to the anoxic episode.

The mean oximeter reading of the group was 76.4 per cent. None of the 50 subjects experienced relapses within 7 days of the chamber flight. Eight of the subjects, however, had relapses at periods varying from 8 to 35 days following the flight. None of the subjects included in the series had positive blood smears before entering the chamber or during the succeeding 5-day period when daily blood examinations were made. Data pertaining to the 8 subjects who relapsed subsequent to the 7-day period are shown in Table 1.

TABLE 1

Data on 8 Cases Which Relapsed Later Than 7 Days After the Anoxic Experience

Case No.	No. of previous attacks	Days since last attack	Days since last atabrine	Relapses		
				Days after chamber flight	Type	Days after last attack
3	7	25	17	8	Vivax	33
6	2	36	31	8	..*	42
8	6	12	3 (quinine)	23	..*	33
13	4	33	17	18	Vivax	51
42	9	26	15	30	..*	56
43	2	26	18	35	Vivax	61
44	5	52	46	17	Vivax	69
49	7	42	35	18	Vivax	60

* Type not reported.

In summary, neither relapse nor parasitemia was observed in a group of 50 individuals giving histories of recent malaria within a period of 7 days following exposure to the anoxia produced by a 1-hour stay at 18,000 feet in a low-pressure chamber.

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