the perpendiculars from P to the K-bounding flats represent the zygotic proportions x_{ii}, $2x_{ij}$, etc., whose sum is unity. Eq. (3) or (4) defines the surface within the simplex on which all equilibrium population points lie.

Eq. (4) also furnishes us a ready method of estimating F from random samples. Let a_{ii} and $2a_{ij}$ be the observed numbers of A_iA_i and A_iA_j, $n_i = \sum_j a_{ij}$ (all j) and $N = \sum_i n_i$, the total number of individuals in the sample. Our sample estimate of F is then

$$f = \frac{1}{k-1}\left\{\sum_i \frac{a_{ii}}{n_i} - 1\right\}.$$

When $k = 2$, this estimate reduces to

$$f = \frac{a_{11}}{a_{11}+a_{12}} + \frac{a_{22}}{a_{12}+a_{22}} - 1,$$

which is identical with

$$f = \frac{a_{11}a_{22} - a_{12}^2}{(a_{11}+a_{12})(a_{12}+a_{22})},$$

as given by Haldane (*4*) and Li (*5*). Other methods of estimating F will be discussed elsewhere.

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The Reaction of Diazonium Salts with Alcohols

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The most remarkable thing about this "well-known" reaction is that it continues to be completely misunderstood in a great many quarters. Griess (*1*) in 1864 reported that benzene diazonium sulfate or nitrate reacts with ethanol to give benzene and acetaldehyde:

$$\text{Aryl—N}_2^+ X^- + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{Aryl—H} + \text{CH}_3\text{CHO} + \text{N}_2 + \text{H}X.$$

This came to be regarded as a general reaction despite the fact that numerous examples of an alternative process, replacement of the $—N_2^+$ by $—OCH_2CH_3$, were soon found:

$$\text{Aryl—N}_2^+ X^- + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{Aryl—OCH}_2\text{CH}_3 + \text{N}_2 + \text{H}X.$$

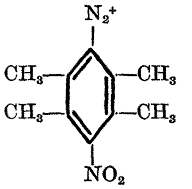
In 1887, it was shown by Remsen and Orndorff (*2*) that Griess had been in error even in the case of benzene diazonium sulfate and nitrate. These react with ethanol to give phenetole contaminated with a little benzene.

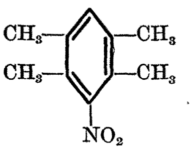
In 1901, Hantzsch and Jochem (*3*) made a very careful study of the action of absolute ethyl alcohol on benzene diazonium chloride and benzene diazonium sulfate. Confirming Remsen and Orndorff, they found that phenetole is the main product, along with a very small quantity of benzene; e.g.:

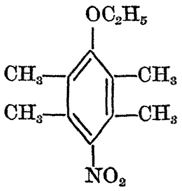
$$\text{C}_6\text{H}_5\text{—N}_2^+\,\text{Cl}^- + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \underset{(61\%)}{\text{C}_6\text{H}_5\text{OCH}_2\text{CH}_3} + \underset{(5\%)}{\text{C}_6\text{H}_6}$$

When they treated benzene diazonium salts with methyl alcohol the methyl ether (anisole) was formed in 70% yield; there was no evidence of benzene formation. Thus, with *either* ethanol or methanol, benzene diazonium salts give ethers as the major product.

Despite the numerous demonstrations of the unreliability of ethanol as a reagent for replacing the diazonium group by hydrogen, its use persisted. Cain (*4*), in 1909, reporting on the reaction of I with ethanol, was so confident that the product was nitrodurene, II, that he did not have it analyzed. Not until 1952 was it recognized that Cain's "nitrodurene" was, in fact, the ether, III (*5, 6*).


I


II


III

(Special interest attaches to this case because nitrodurene was employed in an early test of the concept of steric inhibition of resonance; the sample used had been prepared according to Cain and was actually the ether, III.)

In recent years the view that treatment of diazonium salts with ethyl alcohol results in replacement by hydrogen has been given renewed importance by totally incorrect statements concerning the course of the reaction of benzene diazonium chloride with ethyl alcohol. Incredible as it may seem, the paper by Hantzsch and Jochem (*3*) is cited as the authority for these statements. Thus, a review (1937) reads:

> The reactions of benzene diazonium chloride with methyl and ethyl alcohols are to an appreciable extent qualitatively different, as shown by Hantzsch and Jochem:
>
> $$\text{C}_6\text{H}_5\text{N}_2\text{Cl} + \text{CH}_3\text{OH} \rightarrow \text{C}_6\text{H}_5\text{OCH}_3 + \text{N}_2 + \text{HCl}$$
> $$\text{C}_6\text{H}_5\text{N}_2\text{Cl} + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{C}_6\text{H}_6 + \text{CH}_3\text{CHO} + \text{N}_2 + \text{HCl}.^2$$

Numerous statements made in subsequent years are even less guarded and are exemplified by the following excerpt from a well-known monograph (1949):

> It had previously been shown by Hantzsch and Jochem that the reactions with methyl and ethyl alcohols take different paths.
>
> $$\text{C}_6\text{H}_5\text{N}_2\text{Cl} + \text{CH}_3\text{OH} \rightarrow \text{C}_6\text{H}_5\text{OCH}_3 + \text{N}_2 + \text{HCl}$$
> $$\text{C}_6\text{H}_5\text{N}_2\text{Cl} + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{C}_6\text{H}_6 + \text{CH}_3\text{CHO} + \text{N}_2 + \text{HCl}.^2$$

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[1] Fellow of the John Simon Guggenheim Memorial Foundation and the United States Educational Commission in the United Kingdom, 1952–53.

[2] It would be invidious to give the references to these two quotations since they are but examples of a generalized situation.

In 1944, a correct account of the reactions of diazonium salts with alcohols was published in *Organic Reactions* (*7*), but it has apparently been overlooked by the majority of those who have since discussed this reaction.

In conclusion, two points should be emphasized: (1) ethyl alcohol is not, in general, dependable for replacing diazonium groups by hydrogen (*7*); (2) reduction with hypophosphorous acid is a reliable way to replace diazonium groups by hydrogen (*7, 8*). A second general method for replacing diazonium groups by hydrogen has just been described by Roe and Graham (*9*).

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Biosynthesis of Estrone and β-Estradiol in the Perfused Ovary[1]

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Zaffaroni, Hechter, and Pincus (*1*) found that the adrenal gland converts C^{14}-labeled acetate into adrenal hormones when it is perfused with a solution of this salt. Brady (*2*) showed that testis slices are capable of synthesizing testosterone from C^{14}-acetate, and Savard, Dorfman, and Poutasse (*3*) demonstrated conversion of labeled acetate to testosterone and Δ^4-androstene-3,17-dione in the perfusion of human testis. It therefore seemed interesting to investigate whether the ovary may synthesize estrone or β-estradiol from the same starting material.

The experiment was carried out by perfusing sow ovaries with sodium acetate labeled with C^{14} in the carboxyl group. The technique, including radioactivity counts, and the apparatus were the same as described in an earlier investigation (*4*).

Two perfusions of long duration were carried out with ovaries obtained from one pregnant and one nonpregnant sow. The animals each weighed about 200 lb. Two thousand units of heparin were injected intravenously before beginning the operation to obtain the ovaries. The large and small intestines were removed, and the aorta was litigated just below the origin of the ovarian arteries. The ovaries were freed of all adnexa. The aorta was then cut just above the origin of the ovarian arteries, and the entire specimen mounted in the perfusion apparatus. (As to the apparatus and procedure, *cf.* [*4*].)

The first experiment was carried on for 44 hr. During this time 1000 RU of gonadotrophin and 0.1125 mc of acetate labeled in the carboxyl group were added at 20 min, 9 hr, and 25 hr after circulation in the perfusion apparatus started. At the 9- and 25-hr intervals the perfusing medium was changed. The organ was without circulation for 30 min prior to beginning the perfusion.

In the second experiment, which lasted 13 hr, one injection of 1200 RU of gonadotrophin and 0.225 mc of labeled acetate was made 7 min after circulation was reinstated; the interruption of the circulation lasted 21 min.

The perfusion liquid in both experiments was a mixture of pig's blood and White's solution as described before (*4*). In each experiment the approximate volume of perfusate charging the system was 500 ml.

The weight of the ovaries in the first experiment was 6.1 g, in the second, 3 g. In both instances the organ appeared to have been well circulated by the perfusion liquid. No gross response to the gonadotrophin was evident, but there was also no significant degeneration discernible.

Extraction of the perfused ovaries. Immediately after the perfusion was terminated the mixture of ovaries and perfusion liquid was converted into a mash in a Waring Blendor. One third of the total volume of chloroform was added and again blended for about 10 min. The homogenous mixture was centrifuged at about 2000 rpm in the cold room. Three easily separable layers were formed; the lowest was a clear chloroform solution. The middle layer could be easily separated and consisted mostly of protein. This was stirred with acetone, and the acetone solution was filtered from the insoluble residue, which was again treated with acetone. The combined acetone extracts were evaporated to dryness and extracted with pentane. This solution was added to the chloroform layer. The uppermost layer from the centrifugation was a water solution which was extracted with chloroform. This extract was also added to the main choloroform portion. The combined extracts were then taken to dryness. The fatlike residue was boiled with methanol, and after cooling the clear methanol solution was decanted from the solid residue. This operation was repeated three times. The methanol extracts were evaporated to dryness and divided into two equal portions.

a) Isolation of estrone. The first part of the methanol extract was dissolved in about 10 ml methanol, and 200 mg of pure estrone was added and dissolved by warming. In the cold the estrone crystallized out

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