volving none of the nitrogen fixed in air) of the same magnitude as the fixation in the $N^{15}$-excess atmosphere had occurred, the distribution of molecules in the final atmosphere would have been: mass 30, 2.85 per cent.; mass 29, 12.50 per cent.; and mass 28, 84.55 per cent. These shifts are approximately eight times as great as the observed shifts in equilibrium.

If there had been an exchange between the fixed nitrogen of the culture, which was high in $N^{14}$ from its initial period of fixation in air, with the nitrogen of the atmosphere, this would have been reflected in an approach toward equilibrium and in an increase in the molecular species of masses 28 and 29. If there had been a disruption of the $N_2$ molecules at the seat of fixation and a return of a portion of this nitrogen to the gaseous phase, the effect would have been evident merely as a shift toward an equilibrium condition.

It is evident, however, that the observed changes in the ratios of the molecular species are well within experimental error; and the lack of significance of these slight shifts is further emphasized by the fact that they are all away from equilibrium.

It is unlikely that an exchange reaction will interfere with the use of $N^{15}$ as a tracer for studies of biological nitrogen fixation, but it would be well to test this point with each agent which gives evidence of positive fixation.

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## SEASONAL FLUCTUATION IN ESTROGEN EXCRETION[1]

SEASONAL periodicity in the lower animals is well known, but its recognition as a general principle applicable to the higher mammals has been slow. One of us (H. H. D., 1924, unpublished), experimenting with the pasteurization of cows' milk, found that the degree of separation of cream produced by a given rate of heating varied throughout the year, with a sharp change in the spring; not until 1938 was it shown (Ritzman and Benedict[2]) that the basal metabolism of the cow fluctuates seasonally. The data to be reported here have revealed a marked seasonal periodicity of estrogen excretion in the human female.

In April, 1939, in a series of pooled urines from young non-pregnant women, we found the surprisingly high estrogen content of 1350 I. U. per liter. An Ascheim-Zondek pregnancy test was done on the urine, with negative results. Since figures of this magnitude in the absence of pregnancy had not been observed at other times of the year, a series of experiments was planned for a continuous record of estrogen excretion throughout the year. For this the cooperation of two normal women was obtained.

Owing to the difficulties of continuous collection of specimens, certain days were selected in each menstrual cycle to be tested. In Table 1 are given the results on subject F. A. for the months of August, January, February, March, April and May. It will be seen that January and February gave similar figures, which were markedly lower than those of the preceding August; there then followed a rise in March, and a much greater one in April, with a slight recession in May.

TABLE 1

DAILY EXCRETION OF ESTROGEN IN I. U. DURING VARIOUS MONTHS
(Subject F. A.)

| Day of cycle | Aug. | Jan. | Feb. | Mar. | Apr. | May |
|---|---|---|---|---|---|---|
| 5 | 250 | 30 | 20 | 30 | 200 | 180 |
| 10 | 330 | 150 | 100 | 200 | 250 | 400 |
| 13 | 550 | 270 | 300 | 650 | 850 | 450 |
| 17 | 550 | 50 | ... | 30 | 450 | 400 |
| 22 | 170 | 75 | 170 | 180 | 300 | 250 |
| 25 | 150 | 50 | 50 | 90 | 170 | 350 |
| 6 day total | 2,000 | 625 | > 640 | 1,180 | 2,220 | 2,030 |

Realizing the importance of a closer check on the spring rise, we had increased the number of specimens collected in March, April and May. Fourteen daily specimens in each cycle of 26 days were assayed during these months. The estrogen level was consistently higher in April than in March, whereas May readings were intermediate. The data are given in Table 2.

TABLE 2

DAILY EXCRETION OF ESTROGEN IN I. U. DURING THE SPRING MONTHS, 1940
(Subject F. A.)

| Day of cycle | March | April | May |
|---|---|---|---|
| 5 | 30 | 200 | 180 |
| 10 | 200 | 250 | 400 |
| 11 | 200 | 950 | 400 |
| 12 | 330 | 580 | 550 |
| 13 | 650 | 850 | 450 |
| 14 | 300 | 550 | 250 |
| 15 | 100 | 450 | 350 |
| 17 | 30 | 450 | 400 |
| 19 | 170 | 450 | 450 |
| 21 | 280 | 270 | 350 |
| 22 | 180 | 300 | 250 |
| 23 | 100 | 170 | ... |
| 24 | 90 | 70 | ... |
| 25 | 90 | 170 | 350 |
| 14 day total | 2,750 | 5,710 | > 4,080 |

The second case investigated gave a similar picture. The rise in March was not as striking as that in Subject F. A., but a large rise in April was recorded. Table 3 gives the results of this second case.

The above tables indicate that there is a marked seasonal fluctuation in the estrogen output of normal females. The human, from this evidence, is not exempt from those seasonal influences which operate

[2] National Research Council Fellow.

[1] This work was done under a grant from the Carnegie Corporation, New York.

[2] E. G. Ritzman and F. G. Benedict, "Nutritional Physiology of the Adult Ruminant." Carnegie Institution of Washington, Washington, D. C., 1938.

TABLE 3
EXCRETION OF ESTROGEN IN I. U. DURING VARIOUS MONTHS, 1940
(Subject E. M.)

| Day of cycle | Jan. | Feb. | Mar. | Apr. |
|---|---|---|---|---|
| 5 | 50 | 30 | 20 | ... |
| 6 | 110 | 120 | 100 | 500 |
| 10 | 230 | 350 | 380 | 750 |
| 15 | 40 | 20 | 150 | 100 |
| 20 | 220 | 75 | 200 | 200 |
| 24 | 70 | 20 | 120 | 180 |
| 6 day total | 720 | 615 | 970 | > 1,730 |

elsewhere in the animal kingdom. It is more than ever difficult to state, unless the annual fluctuations are taken into account, what is the normal output of estrogen by a woman.

A more detailed report of this investigation will be published shortly in *Endocrinology*.

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## THE EFFECT OF TYROSINASE ON ARTERIAL HYPERTENSION

BECAUSE the substance responsible for some varieties of arterial hypertension may be a simple amine, particularly one containing a phenolic group, a pure preparation of tyrosinase, a phenolic oxidase obtained from mushrooms by Dr. J. M. Nelson, was used in animals exhibiting "renal" hypertension. It was found that tyrosinase is effective in lowering raised arterial pressure in rats and dogs when their kidneys are injured.[1,2] The pressor substance may accordingly contain a phenolic group.

Further evidence for this theory was obtained by ascertaining the effect of tyrosinase upon certain pressor substances. It was found that renin is inactivated by tyrosinase when catechol is present—probably through the mediation of the orthoquinone formed. Angiotonin, obtained from Dr. Irvine H. Page, is also inactivated by tyrosinase when serum is present, suggesting that angiotonin, in its active state, contains a phenolic configuration in the molecule. The pressor substance obtained from the anaerobic autolysate of kidneys, prepared by Dr. Joseph Victor, is inactivated directly by tyrosinase, as is, of course, adrenalin and tyramine.[3]

Since the results in animals were satisfactory, it appeared necessary to ascertain the effect of this enzyme upon hypertension exhibited by human beings. Seventeen patients suffering from arterial hypertension have been treated by daily subcutaneous injections of varying amounts of tyrosinase for three to four weeks. In fourteen the systolic pressure had been persistently above 200 mm Hg and the diastolic above 120. In all but one the blood pressure fell a significant amount; in seven to 140 to 160 mm Hg systolic, and 80 to 100 diastolic, and in six to 160 to 180 systolic and 100 to 115 diastolic. In the other three, the response was less. Three patients in a late stage of the disease were improved. In one there was no effect.

The fall in blood pressure was accompanied by certain other changes, indicating that a general effect upon the disease had occurred. In seven patients whose electro-cardiograms were altered a change in the direction of normal occurred. In three the hearts became small as observed in x-ray photographs. In all but one the level of the urea nitrogen in the blood was lowered, but the clearance of urea was unaffected. Symptoms, when present, were relieved. In four, hemorrhagic and exudative lesions were present in the eyegrounds. These disappeared. No change in the ability of the kidneys to concentrate urine was observed.

When injections of tyrosinase were stopped, the blood pressure soon (within three to six days) returned to its previous level. Symptomatic improvement, as well as the improvement in the ocular fundi, lasted for several weeks or months.

Injections were painful at times; at others no discomfort occurred. Occasionally a moderate degree of pyrexia followed the injections. Allergic reactions at the site of injection developed in three patients.

On one occasion a small amount of enzyme was given intravenously. This was followed by a severe reaction, with nausea, vomiting, signs of increased peristalsis, fall of blood pressure and bradycardia. The blood pressure remained low for twenty-four hours afterward. Significant decrease in the clearance of urea did not occur. Although the blood pressure fell from 220 mm Hg systolic and 150 diastolic to a level of 130 systolic and 90 diastolic for this period, the patient remained comfortable.

Relatively large doses were needed when given by the subcutaneous route. Since the enzyme is a protein, it is doubtful whether absorption was complete. Deposition of grey or yellow pigment at the site of injection was a common occurrence.

It is evident that subcutaneous injections of tyrosinase effectively but only temporarily lower blood pressure in certain cases of arterial hypertension in human beings. These results suggest that some phenolic substance is altered.

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