The state societies of entomologists and taxonomists held their meetings on Saturday.

The Junior Academy, composed of 38 high-school science clubs, met on Saturday with 250 young scientists present. Addresses were given by Walter C. Geisler, Shortridge High School, Indianapolis, on "A New Technique in Gem Cutting," and L. S. Shively, Ball State Teachers College, on "Astronomy." Eleven papers were given by members of the Junior Academy. The following officers were chosen for 1941: *President,* Patricia Anderson, Edison High School, Hammond; *Vice-president,* Mary Hybarger, Lew Wallace High School, Gary; *Secretary,* Mary Lou Sweet, Marion High School. Honorary memberships in the American Association for the Advancement of Science were awarded to Frances Scott, Arsenal Technical High School, Indianapolis, and Robert Gericke, Lew Wallace High School, Gary. Dean Howard E. Enders, Purdue University, senior academy sponsor of the Junior Academy, closed the meeting with a report on the progress of the Indiana Junior Academy through the state.

WILL E. EDINGTON,
Press Secretary

SPECIAL ARTICLES

APPLICATION OF N^{15} TO THE STUDY OF BIOLOGICAL NITROGEN FIXATION

STUDY of the mechanism of biological nitrogen fixation should be greatly aided if isotopic nitrogen could be used for tracing the path of nitrogen from its molecular to its fixed state in the cell. Inaccuracies of the Kjeldahl method have frequently suggested fixation of nitrogen by germinating seeds, Rhizobium independent of its host, non-leguminous plants and other biological agents whose ability to fix nitrogen is questionable. It would be possible to detect nitrogen fixation unequivocally, however, by the appearance of excess N^{15} in a biological agent under an atmosphere containing excess N^{15}, provided no direct exchange of N^{14} and N^{15} occurred between the fixed and the gaseous nitrogen in the system.

A culture of the free-living, nitrogen-fixing organism *Azotobacter vinelandii* was used to test for exchange. Nitrogen gas containing 35 per cent. N^{15} excess was mixed with air to produce a non-equilibrium condition of the molecular species of N_2. This gas mixture was introduced into an evacuated culture vessel containing 30 ml of a three-day culture of *Azotobacter vinelandii* which had grown in air and fixed 117.7 micromols N_2 (as shown by Kjeldahl analysis). In four more days the culture fixed 150.2 micromols additional N_2 under the N^{15}-excess atmosphere. Samples of gas and culture were taken at the time the culture was first supplied with the N^{15}-excess atmosphere and at the termination of the experiment.

By assuming non-selective fixation and no exchange, it was possible to calculate (from the composition of the two atmospheres supplied to the culture and the Kjeldahl analyses) the final N^{15} content of the culture as follows:

		Micromols N^{15}
117.7 micromols N_2 fixed in air (0.37% N^{15})		0.43
150.2 micromols N_2 fixed in N^{15}-excess atmosphere (9.12% N^{15})		13.70
267.9 Total	Total	14.13

$$\frac{14.13 \text{ micromols } N^{15}}{267.9 \text{ micromols } N_2 \text{ fixed}} = 5.27\% \ N^{15}$$

Mass spectrographic analysis indicated a final concentration of 5.23 per cent. N^{15} in the culture.

The close agreement between the calculated and observed values indicates that there was no selective action in the fixation of N^{15} and N^{14} atoms from the molecules of mass 28, 29 and 30, and that there is no apparent exchange reaction between molecular nitrogen and fixed nitrogen in the culture. Schoenheimer and Rittenberg[1] have presented convincing evidence that the animal body exerts no selection between N^{14} and N^{15} in combined forms, but our study seems to provide the first direct extension of that observation to a biological process involving molecular nitrogen.

Analysis with the mass spectrometer indicated that initially the N^{15}-excess atmosphere contained 9.12 per cent. N^{15} and finally contained 9.11 per cent. N^{15}. The analysis showed the distribution of molecular species as in Table I.

TABLE I

Molecule		Concentration as percentage of total	
Composition	Mass	Initial	Final
N^{15}_2	30	2.99	3.01
$N^{15}\,N^{14}$	29	12.24	12.20
N^{14}_2	28	84.77	84.79

Calculations show that at equilibrium between the molecular species the distribution would have been as in Table II.

TABLE II

Molecule		Concentration as percentage of total	
Composition	Mass	Initial (9.12% N^{15})	Final (9.11% N^{15})
N^{15}_2	30	0.83	0.83
$N^{14}\,N^{15}$	29	16.58	16.56
N^{14}_2	28	82.59	82.61

Of the N^{15}-excess atmosphere supplied, 6.4 per cent. was fixed by the organism. If an equilibration (in-

[1] R. Schoenheimer and D. Rittenberg, *Physiol. Rev.*, 20: 218, 1940.

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.