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Incorporation of Arsenic into Cock Sperm.* (29581)

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Arsenicals have been shown to stimulate growth and improve feed efficiency in broilers and to improve laying hen performance. Although Pope and Schaible(1) reported that arsanilic acid exerts a sparing action on the protein requirement of breeding hens and layers when egg production was the criterion, small amounts did not improve hatchability or fertility in breeding chickens. Excess arsenic has been shown to be toxic to chickens. The position of arsenic in the periodic table would suggest that it has some chemical properties which are similar to phosphorus, and therefore, could replace phosphorus in some compounds. Since $P^{32}O_4$ or phosphate-P^{32} is readily incorporated into the deoxyribonucleic acid (DNA) of cock sperm in amounts allowing its detection(2) it was decided to use radioactive Arsenic (As^{76}) and also stable arsenic in mineralization studies with cock sperm to determine if arsenic was incorporated into the DNA of sperm.

Experimental. Ten sexually active New Hampshire cocks selected from a group maintained for artificial insemination were each given 200 μc of radioactive $Arsenic^{76}$ as As_2O_3 subcutaneously. The semen was collected 26 hours after radionuclide administration and each 26 hours thereafter for 4 collections. Twenty-six hours was used as the collection time because it was estimated that this period plus the time required to fractionate the semen would be equivalent to one half-life of $Arsenic^{76}$ (about 26.8 hours). The radioactivity of the semen, sperm, seminal fluid, protein (TCA precipitate) and DNA fraction was determined. The DNA fraction was prepared as outlined by Borenfreund, Fitt and Bendich(3). The results are shown in Table I and demonstrate that $Arsenic^{76}$ was readily incorporated into cock sperm. Most of the $Arsenic^{76}$ was found in the diluent (seminal fluid) and fractionation of the sperm showed that on the 52 hour period the sperm contained the greatest concentration of $Arsenic^{76}$. The DNA fraction had its greatest concentration on the 78 hour period and protein fraction on the 52 hour period.

Since these data indicated that $Arsenic^{76}$ was in the DNA fraction of sperm, a further study was designed to study the incorporation of stable arsenic into the DNA of sperm. Twenty-four cocks were placed on a breeding ration containing 500 g of arsanilic acid per

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TABLE I. Distribution of As^{76} in Cock Sperm.*

Fraction	Day 1/26 hr	Day 2/52 hr	Day 3/78 hr	Day 4/104 hr
Semen	150	150	116	32
Sperm	30	56	48	21
Diluent†	120	82	46	16
Protein‡	4.2	22	16	6
Ether-alcohol fraction§	12	16	12	7.2
DNA fraction	8	14	18.8	7.6

* All results (corrected for radio-decay) are expressed as counts per minute (cpm) $\times 10^2$.
† Diluent consisted of seminal fluid plus the buffer used to wash the sperm.
‡ Trichloroacetic acid precipitate.
§ Ether-alcohol (50-50) soluble fraction.

ton of ration. After receiving this ration 10 days, the cocks were placed on a non-arsenic supplemented breeding ration and the semen collected twice weekly for 4 weeks. The semen collected each time was mixed, a 1 ml aliquot of semen taken, and the DNA removed and purified by the method previously used. The reprecipitated DNA from each aliquot prepared from each collection was placed on ash free filter paper discs and dried. Duplicate samples were prepared from each collection. The samples were placed in a plastic tube along with standards containing As_2O_3 and subjected to neutron activation in a 75 watt reactor fueled with enriched uranium oxide. The $Arsenic^{76}$ was determined immediately after neutron activation and also again 26.8 hours later with a single channel analyzer. The results obtained on the 26.8 hour period are shown in Fig. 1. The results demonstrate that arsenic is incorporated in the DNA. Furthermore, it appears to be incorporated into the chemical structure at about the same time rate as phosphorus because the greatest concentration of arsenic appears at a comparable time to $P^{32}O_4$ maximum concentration(2).

The peak at 3 days may have been due to adsorbed arsenic which had a weak bond to the structures in the nucleus of the sperm.

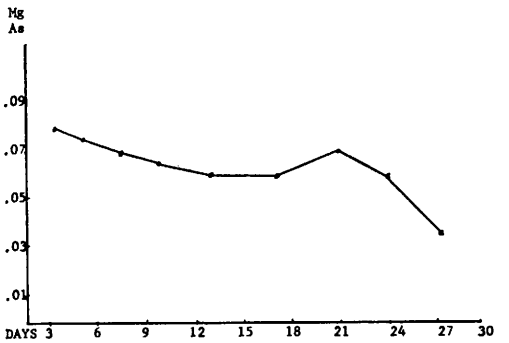

FIG. 1. Arsenic content of DNA from cock sperm.

These data do not predict the location of arsenic in the DNA but since reprecipitation of the DNA did not result in a decrease in arsenic, it was assumed that the arsenic was bound and not adsorbed.

These findings show that arsenic forms arsenoproteins and arsenolipids and it is placed in the DNA of cock sperm.

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