Technical Papers

The Influence of X-Irradiation and Atabrine Upon Feathering in the Chick[1]

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Mushett and Siegel (*1*) have described the hematological changes induced by large doses of Atabrine [3-chloro-7-methoxy-9-(1-methyl-4-diethylaminobutylamino)acridine dihydrochloride]. Although they did not observe a leucocytosis in the chicken, this change has been observed in this laboratory (*2*).

In a study designed to investigate modifications of the x-irradiation-induced leucopenia in the chick, it has been observed that feathering is inhibited by irradiation and that this inhibition is overcome by adding Atabrine to the diet.

FIG. 1. Chick on the left received the purified diet supplemented with Atabrine; chick on the right received no Atabrine.

Day-old White Rock chicks were divided into two groups of 15 chicks each and housed in a metal brooder. One group received the purified diet described by Keith *et al.* (*3*), supplemented with 2 mg folic acid/kg of diet. The other group received this same diet plus 500 mg Atabrine dihydrochloride/kg of diet. Food and water were given *ad lib.* After 15 days on the respective diets the chicks were irradiated using the following factors: 15 ma, 220 kv, inherent filtration only, HVL 0.25 mm Cu, 32 in. to the center of the animal, 20.4 min at a rate of 42.5 r/min to a total of 865 r (in air), field size covering entire chick.

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The average initial weights, as well as the average rate of weight gain of the chicks over the experimental period, were equal.

The chicks were photographed 26 days after irradiation, one bird from each group being selected at random for each photograph. At approximately 15 days after irradiation it was observed that the chicks that had received Atabrine were feathering at a faster rate than those not receiving Atabrine. The birds that had not received Atabrine produced apparently normal wing and tail feathers, but they developed very few feathers on the breast, back, and neck (Fig. 1).

Further studies are in progress and will be reported at a later date.

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Does Phosphorylated Hesperidin Affect Fertility?[1]

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Phosphorylated hesperidin, a hyaluronidase inhibitor (*1*), has recently been reported by several investigators to act as a new antifertility factor when administered either orally or intraperitoneally to the rat (*2*), mouse, and human (*3*). Since it is claimed that this substance may be used orally in both males and females, such an important discovery deserves further critical verification. Moreover, if this substance has an effect on fertility, it is of importance to determine whether this is due to the inhibition of sperm penetration into the eggs, as interpreted by these investigators, or whether it is due to the inhibition of ovulation, of the physiological functions of the germ cells, or of implantation and normal development of the embryo. Adult rats and rabbits were used in the present experiments to evaluate these possibilities.

The following two series of tests were performed on rabbits:

a) In order to determine whether phosphorylated hesperidin inhibits fertilization, a doe was bred twice by fertile bucks and 10½–11 hr later, at the time when pene-

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tration of sperm into the eggs normally occurs, .03 ml 1% phosphorylated hesperidin in saline was deposited via the fimbria into the ampulla of each Fallopian tube. The animal was sacrificed 24 hr after operation, and the eggs were recovered, fixed, stained, and examined to determine whether they were fertilized (*4*).

b) In order to investigate the possibility that phosphorylated hesperidin inhibits the functional activity of spermatozoa, semen collected with an artificial vagina was suspended in saline containing different concentrations of phosphorylated hesperidin. A 0.5 ml aliquot (containing 5–8 million spermatozoa) of one of these suspensions was deposited in the vagina of female rabbits, and pituitary extract was injected intravenously to induce ovulation. The animals were sacrificed 24 hr later, and the eggs were recovered, fixed, stained, and examined. The results of these two series of tests are presented in Table 1.

TABLE 1

FERTILIZATION OF RABBIT EGGS

	Concentration (%)	Rabbit	No. of eggs	Eggs fertilized (%)	Condition of fertilized eggs
Deposition of phosphorylated hesperidin into the tubes	1	1	15	100	4–8 Cell stage
		2	9	100	
		3	4	100	
		4	13	100	
Insemination of sperms suspended in phosphorylated hesperidin	1	5	7	0	Pronuclear, or 2-cell, stage
		6	13	15	
		7	3	0	
		8	11	55	
	.1	9*	55	42	Pronuclear, or 2-cell, stage
		10	11	36	
		11	11	100	
		12	11	64	

* Superovulated.

Two experiments were performed on rats in the following manner. (The dosage used was approximately the same as stated by previous investigators (*2, 3*)—i.e., 20 mg/kg intraperitoneally and 100 mg/kg orally.)

a) Ten females, of 200–250 g body weight, and 6 males of proved fertility were each injected intraperitoneally daily for 6 days with 5 mg of phosphorylated hesperidin in saline. Then two groups of 5 females and 3 males were put together in separate cages, and injections were continued until the females were killed.

b) Another 11 females and 6 males were hand-fed daily with 20 mg of phosphorylated hesperidin in 0.5 ml of saline. Six days later, 5 females and 3 males were put together in one cage, and 6 females and 3 males in another cage. After two weeks of hand-feeding, in order to avoid further emotional disturbance of the animals, 20 mg of phosphorylated hesperidin was dissolved in 10 ml of water and served as drinking water daily for each animal until the end of the experiment.

In both experiments, the animals were killed either when pregnancy was assured by palpation or 27 days after the beginning of the experiment. The numbers of corpora lutea and those of normal and degenerated embryos were recorded. The ages of the embryos were determined by their size in order to estimate the time of conception. The results of these two tests and data collected in control animals are presented in Table 2.

TABLE 2

PREGNANCY OF RATS

	No. of female rats		Av time interval between cohabitation and conception (days)	Av no. of corpora lutea	Av no. of normal embryos	Av no. of degenerated embryos
	Not pregnant	Pregnant				
Intraperitoneal injection of phosphorylated hesperidin (5 mg/rat/day)	0	10	5.4	13.6	10.9	.5
Oral administration of phosphorylated hesperidin (20 mg/rat/day)	2	9	4.5	12.5	10.5	.33
Control	0	9	5.2	8.7	6.4	.2

From the results of this study, it is quite clear that phosphorylated hesperidin does not inhibit fertilization when deposited into the Fallopian tubes of rabbits at the time of sperm penetration, nor does it inhibit ovulation, implantation, or normal development of the embryo when administered intraperitoneally or orally to rats. Although the motility was unaffected, however, the fertilizing capacity of rabbit sperm appeared to be inhibited to a certain extent when the sperm was suspended in a 1% solution of phosphorylated hesperidin. That such a concentration of phosphorylated hesperidin could reach the testis or epididymis of an animal when administered orally or intraperitoneally is very doubtful. It should be mentioned here that, although a 1% solution of phosphorylated hesperidin can inhibit the fertilizing capacity of the spermatozoa *in vitro*, it does not inhibit fertilization or even the fertilizing capacity of the spermatozoa *in vivo* when deposited into the tubes.

Since there is so great a discrepancy between the results obtained in the present study and those reported by previous workers, it was thought that the potency of the phosphorylated hesperidin as a hyaluronidase inhibitor should be determined. Therefore, one group of rabbit eggs in mucous clots, recovered 10½ hr after a sterile mating, was placed in a watch glass containing 1 ml of a 0.01% solution of phosphorylated hesperidin in saline, and another group in one

containing saline alone. Then .05 ml of undiluted rabbit semen was added to each watch glass. Periodic observation revealed that the dispersal of the cumulus clot took 10 min in the saline solution, and ½–1 hr in the phosphorylated hesperidin. Therefore, the phosphorylated hesperidin used in the present study was undoubtedly potent as a hyaluronidase inhibitor, but its potency is relatively weak as compared with nitrated hyaluronic acid (*5*) in the inhibition of follicular cell dispersal and in the inhibition of the fertilizing capacity of spermatozoa.

Our knowledge of the actual function of sperm hyaluronidase in fertilization is as yet obscure, but we do know that one of its functions is the dispersal of follicular cells surrounding the egg. However, since it has been shown by several investigators that follicular cell dispersal is not a prerequisite of sperm penetration into the eggs (*6*), a hyaluronidase inhibitor would not necessarily be a fertilization inhibitor.

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The Natural Concentration of Deuterium in Honey[1]

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In the early 1930s, when heavy hydrogen excited the interest of scientists, extensive studies were carried out with the application of deuterium to biological systems.

The enthusiasm resulted in interesting findings by Hevesy, Schoenheimer, Rittenberg, Dole, Washburn, DeWitt-Stetten, and others, yet many problems were left unsolved and many phenomena unexplained.

It has been found that living organisms will tolerate only to a certain limit the exchange of the water in the body to the chemically identical deuterium oxide. Experiments on this problem resulted in the hypothesis that deuterium, having a different resonance, or exchange potential, from hydrogen toward certain bonds of intermediate metabolites, will interfere with the proper work of the donors or acceptors, as well as other enzyme systems essential for the maintenance of life. In accordance with this theory some of the data indicate that living tissues have a tendency to accumulate deuterium to a limited degree.

The appearance of deuterium in tissues can be easily explained by the uptake of natural water, which contains in general about 1 part of the isotope in 5000 parts (*1*) or, more precisely, 0.0147 mol% D (*2*). But further work is necessary to explain, for example, why the sap of a willow tree is significantly richer in deuterium and why the wood has an increment double that of sap (*1*). Since the vapor pressure of deuterium oxide is about 7% below that of water (*2*), it is possible that through transpiration plants are acting as a still and that by fractioned distillation of large quantities of water they might accumulate deuterium oxide in their tissues.

It is known that hydrogen of the OH-groups in glycogen or sugars can be exchanged for deuterium (*3*) if the latter is in abundance in the environment, but this process is slow (*4*) and would hardly account for the excess of deuterium in metabolites. A more likely theory is that deuterium enters into metabolic processes and becomes bound to carbon atoms (*5*); hence the increment as reported, for instance, in honey (*6*).

Little is known about the physiological effect of deuterium, but since it does interfere with metabolism and it is built into carbohydrate molecules, it is available to every cell or tissue through anabolic reactions. Beyond the known physiological action of deuterium, there might be a long-range effect reaching into the realm of genetics, or the theories of neoplasts.

Density measurements for the determination of the deuterium content were carried out with the falling-drop (*7, 8*) or the totally immersed float method (*9, 10*) or with the Cartesian diver. These methods are very sensitive, yet from many points of view it is more convenient to use a mass spectrometer (*11*).

It was of interest to repeat the experiments with honey, extending them to separate determinations of the deuterium ratio in the moisture and the sugars of honey. The material used was a *i95i* buckwheat honey from the Finger Lakes region, with a moisture content of 17.9%. The following samples were analyzed:

Sample M: The somewhat crystallized honey was dehydrated at room temperature, and the moisture trapped in a cooling mixture of acetone–solid carbon dioxide. The condensed liquid was extracted with ether to eliminate volatile metabolites, then the total amount was redistilled to avoid fractionation of deuterium oxide.

Sample M_2: To eliminate crystal water, the dry honey was heated slowly under normal pressure until the first signs of caramelization. This residual water was condensed and passed through a combustion tube.

Sample S: The slightly caramelized honey was combusted with oxygen and a platinum sponge. The condensed water was recombusted to eliminate all traces of organic matter.

Sample D: The crystals of the honey, mainly dextrose, were separated by vacuum filtration and washed with absolute methyl alcohol.

Sample L: The honey which passed through the Buchner funnel was dried *in vacuo*. It contained 36% more levulose than dextrose. Samples D and L were combusted as described for Sample S.

For the measurements in the mass spectrometer,

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