3. TOVE, S. R., NISS, H. F., and WILSON, P. W. *J. Biol. Chem.*, **184**, 77 (1950).
4. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. *Manometric Techniques and Tissue Metabolism.* Minneapolis: Burgess (1949).

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A Saliva Test for Prenatal Sex Determination

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During the course of investigating some of the many ramifications of the Richardson Pregnancy Test (*1*) two rather interesting observations were made. The Richardson test depends upon the presence of free estrone, in contrast to bound or modified estrone or similar 17-ketosteroids in the female urine. This level of free estrone substances rises sharply soon after conception, the test being positive in as little as 2 weeks after conception, sometimes even before the first missed menstrual period. We were naturally interested in determining whether the free estrone level rose in other body fluids. Blood and urine have already been mentioned in Richardson's original article. We investigated such fluids as saliva, tears, and perspiration. The studies upon saliva yielded the results that are the subject of this report.

It was noted early in the study that in only some of the women who were in their sixth or seventh month of pregnancy did the Richardson test prove positive when the saliva was tested. In each of these cases, however, the test was positive on the urine. The apparent answer to the problem was forthcoming only after the delivery of the child. In nearly every case, the positive tests were associated with a male child, and most of the negative tests were associated with a female child. A detailed study followed. The results are presented in Table 1.

TABLE 1

RELATION BETWEEN SEX OF CHILD AND REACTION OF THE MOTHER'S SALIVA TO THE RICHARDSON TEST

	Positive	Negative
Males	218	3
Females	7	148

The precise nature of the substance responsible for the positive test is not known. It is believed that some androgenic substance is being identified, since, whereas a nongravid female normally yields a negative test, after the injection of testosterone or androsterone a strongly positive reaction results. The male saliva, spermatic fluid, and blood serum are all strong positive reactors.

The selective excretion of certain blood constituents through the salivary gland is well known. These findings illustrate a rather delicate selectivity of female salivary glands in their capacity to screen out certain female-associated hormones, but to allow certain male-associated ones to pass into the salivary fluid.

A detailed report of this project will be published in suitable medical journals. Because of their obvious practical nature, these findings are preliminarily reported here to allow early verification by others.

Reference

1. RICHARDSON, G. C. *Am. J. Obstet. Gynecol.*, **61**, 1317 (1951).

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Influence of Cobalt on Reproduction of Mice and Rats

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Reproduction studies with mice and rats kept on whole plant rations have been in progress in this laboratory for several years. In the first experiments, poor reproduction performance was observed (*1*), but later much more satisfactory results were obtained with a slightly modified diet (*2*). The only difference in the composition of the diets was that the salt mixture supplement used originally did not contain cobalt, whereas in the later experiments 0.2% $CoCl_2$ was added to the salt complement. Because of the apparent influence of this small amount of dietary Co on reproduction, the present study was undertaken.

The rats used were of the Sprague-Dawley strain, raised in our laboratory; the mice were of the same albino strain used in the previous experiments. The rats had been kept on a whole plant ration for at least 3 generations prior to the start of the present experiments, and the mice for at least 5 generations. The animals were kept on the basal ration in common cages until females became pregnant. These were put in single screen-bottomed cages and given the respective experimental diets and water *ad lib.* Litters were reduced by random selection to 6 and weaned at the age of 28 days.

The composition of the basal diet was: solvent-extracted soybean meal, 46; cornmeal, 46; sesame oil containing 0.2% percomorphum oil and 0.2% wheat germ oil, 5; salt mixture II USP, 2; thiamine, 0.3 mg; riboflavin, 0.3 mg; calcium pantothenate, 2 mg; pyridoxin, 0.2 mg; choline hydrocloride, 100 mg; nicotinic acid, 2 mg; folic acid, 0.025 mg; biotin, 0.01 mg; inositol, 10 mg; PABA, 25 mg. The diet has been analyzed by microbiological methods and found to contain about 0.5 μg/100 g of vitamin B_{12} activity (*2*). Cobalt was supplied in the diet by adding 0.2% $CoCl_2$ to the salt mixture, or in the drinking water in a concentration of 0.2 mg% of $CoCl_2$; when cobalt was injected, a 0.1% solution of $CoCl_2$ in physiological saline solution was used, of which 0.2 ml was injected in each female 2–6 days prior to giving birth. A total of 130 litters of mice and 64 litters of rats was used in the present study.

TABLE 1

REPRODUCTION OF MICE, KEPT ON A SOYBEAN-CORN RATION FOR SEVERAL GENERATIONS WITH AND WITHOUT COBALT OR VITAMIN B_{12} SUPPLEMENTS

Group	Supplements	No. litters born	No. litters dead	No. young weaned/ litters born	Wt of young at 28 days (± S.E.$_m$)*	Wt change of mothers (± S.E.$_m$)
1 (Control)	None	42	11	3.4	11.5 ± 0.377	− 1.3 ± 0.341
2	4 mg% $CoCl_2$	20	1	4.7†	11.9 ± 0.482	+ 0.7 ± 0.459†
3	0.2 mg% $CoCl_2$ in the drinking water	28	4	4.5†	11.7 ± 0.327	0 ± 0.408†
4	0.2 mg $CoCl_2$ injected i.p.	21	5	3.0	11.4 ± 0.375	− 0.4 ± 0.650
5	4 μg% Vitamin B_{12}	19	2	5.0†	15.2 ± 0.364†	− 0.5 ± 0.534

* S.E.$_m$ = Standard error of mean.

† Difference from control lot significant at 5% level.

The effect of cobalt on the reproduction of mice was studied in 4 series (Table 1). In the control group receiving the soybean-corn ration without added $CoCl_2$, the number of young weaned/litter born was 3.4. With the addition of 4 mg% $CoCl_2$ to the diet, the ratio of young weaned/litter born was 4.7. When the drinking water contained 0.2 mg% $CoCl_2$, the survival of the young was practically equal to the group receiving the Co supplement in the diet. Intraperitoneal injection of a single dose of 0.2 mg of $CoCl_2$ into the mother before giving birth did not significantly improve the survival of the young in comparison with the control group. Performance of the group receiving vitamin B_{12} in the diet was markedly better than that observed with the Co-supplemented diets.

The results obtained with rats (Table 2) were similar to those observed with mice. Only 1.4 young survived the 4-weeks weaning age out of each litter born on the diet without added Co, whereas on the Co-supplemented diet 1.75 survived, on the average. When Co was added to the drinking water, the corresponding value was 2.9. The mean weights of the young at the age of 28 days were 35, 54, and 55 g, respectively. The group receiving the $CoCl_2$ in the drinking water did as well as a group receiving the corn-soybean diet supplemented with 40 μg/kg of vitamin B_{12}. The supplementation also caused a significant weight gain of the mothers during the suckling period.

The difference between the numbers of litters dead in the control series and in those receiving a supplemented diet was never significant. This may be due to the fact that the feeding of the supplement was started only a few days before the birth of the litters.

The results of these experiments indicate that cobalt is of definite benefit for the reproduction of both mice and rats when they are kept on a whole plant ration low in vitamin B_{12}. The amount of Co present in the diet was not determined. The daily amount of Co derived from the supplement would be approximately 0.4 mg in the diet and 0.02 mg in the drinking water, respectively, for the adult female rats. Underwood (*3*) observed no improved growth in rats ingesting only 0.4 μg/day of Co when the diet was supplemented with this mineral. It has been concluded, therefore, that the normal need of the rat, if any, would not be higher than 0.4 μg/day of Co for growth. Our unsupplemented diet probably provided not less than this amount.

Lately it has been shown that Co forms part of the molecule of vitamin B_{12} (*4*) and that the addition of its salts to the culture medium stimulates the microbiological synthesis of this vitamin (*5*). It therefore seems possible that a stimulation of the intestinal synthesis of vitamin B_{12} is a likely explanation for the results described in this study. The fact that Co administered parenterally had no significant effect lends weight to this explanation, although the effect of prolonged and repeated administration of smaller doses of parenterally administered Co should be studied. It is known that ruminants have a dietary

TABLE 2

REPRODUCTION OF RATS, KEPT ON A SOYBEAN-CORN RATION FOR SEVERAL GENERATIONS WITH AND WITHOUT COBALT OR VITAMIN B_{12} SUPPLEMENTS

Group	Supplements	No. litters born	No. litters dead	No. young weaned/ litters born	Wt of young at 28 days (± S.E.$_m$)*	Wt change of mothers (± S.E.$_m$)
1 (Control)	None	18	11	1.4	35 ± 1.776	0 ± 6.99
2	4 mg% $CoCl_2$	12	8	1.75	54 ± 4.350†	16 ± 2.35†
3	0.2 mg% $CoCl_2$ in the drinking water	21	9	2.9†	55 ± 3.580†	11 ± 2.40
4	4 μg% Vitamin B_{12}	13	4	3.2†	59 ± 5.233†	24 ± 4.67†

* S.E.$_m$ = Standard error of mean.

† Difference from control lot significant at 5% level.

need for cobalt, the relation of which to the internal microbiological synthesis of vitamin B_{12} is still not clear (*6*). Klosterman *et al.* (*7*) have found that Co stimulates the growth of pigs fed a soybean-corn ration, whereas in a recent report (*8*) Co has been stated to be ineffective in speeding the growth of rats fed a diet low in vitamin B_{12} and containing iodized casein. The different techniques used in this study and in our experiments make a comparison of the results difficult, but it seems possible that the effectiveness of Co supplements depends on the special microbiological flora prevalent in the experimental animals.

It should be kept in mind that a considerable part of the human population subsists on diets very poor in animal products and therefore probably low in vitamin B_{12}. In these people the possible dietary need for cobalt should be considered.

References

1. JAFFÉ, W. G. *Z. Vitamin-, Hormon-, Fermentforsch.*, **2**, 493 (1949).
2. ———. *Arch. Biochem.*, **27**, 464 (1950).
3. UNDERWOOD, E. J. *Nutrition Abstracts & Revs.*, **9**, 515 (1940).
4. RICKES, G. M., *et al. Science*, **108**, 134 (1948).
5. HENDLIN, D., and RUGER, M. L. *Ibid.*, **111**, 541 (1950).
6. BECKER, A. E., *et al. Ibid.*, **110**, 71 (1949).
7. KLOSTERMAN, E. W., *et al. Ibid.*, **112**, 168 (1950).
8. TAPAN, D. V., *et al. Arch. Biochem.*, **29**, 408 (1950).

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The Szilard-Chalmers Process in Solid Phosphorus Salts

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The formation of electron-pair bonds is well known in organic systems where a nucleus has a high kinetic energy as a result of a nuclear reaction. In inorganic compounds processes of this type are more difficult to recognize, as it is often possible to explain the formation of polyatomic ions as a result of secondary reactions (*1*). For this reason the formation of radioactive pyrophosphates in different phosphorus salts under neutron irradiation may be of some interest, as pyrophosphate formation does not take place in dilute aqueous solutions.

The solids listed in Table 1 were exposed to slow neutrons. The materials were dissolved in water containing pyrophosphate, orthophosphate, phosphite, and hypophosphite ions and the pyrophosphate was precipitated as zinc pyrophosphate or as cadmium pyrophosphate. From the figures in Table 1 it is evident that the analytical technique has very little influence on the results obtained. The determination of the activities of the different ions is possible because no exchange of phosphorus occurs between orthophosphate and pyrophosphate (*2*) or between orthophosphate and phosphite (*3*).

TABLE 1

Compound irradiated	Ion used for pyrophosphate precipitation	Fraction of total activity found in: Orthophosphate	Phosphate from pyrophosphate	Phosphite + hypophosphite	Phosphite + hypophosphite from pyrophosphate
$Na_4P_2O_7 \cdot 10\ H_2O$	Zn	0.36	0.43	0.19	0.02
	Cd	.35	.43	.20	.02
$Na_4P_2O_7$	Zn	.11	.47	.28	.13
	Cd	.10	.48	.32	.10
$Na_2HPO_4 \cdot 2\ H_2O$	Zn	.22	.18	.51	.08
	Cd	.22	.18	.52	.06
Na_2HPO_4	Zn	.20	.25	.46	.09
	Cd	.26	.22	.46	.05
$Na_2HPO_3 \cdot 5\ H_2O$	Zn	.10	.07	.77	.06
	Cd	0.13	0.06	0.70	0.10

It is evident that radioactive phosphorus might very well occur in other ions than those listed as carriers. Radioactive pyrophosphites, pyrophosphatephosphite and other types must clearly be taken into account, but it is impossible to add carriers for all these species. In our experiments part of these materials may have followed the pyrophosphate and part may have gone with the orthophosphate and phosphite fractions. Therefore not only the filtrates of the pyrophosphate were separated into an orthophosphate and a phosphite + hypophosphite fraction (by ammonium magnesium phosphate precipitation), but the pyrophosphates were dissolved, hydrolyzed, and separated the same way.

The figures, which are (except in the first, second, and fourth lines) averages of two independent irradiations that were in good agreement, show clearly that in all cases an appreciable fraction of the total activity is present as pyrophosphate ions. There are indications that other radioactive ions containing two phosphorus atoms are formed, too.

It should be mentioned that if a pyrophosphate precipitate is not made but hydrolysis is performed, followed by a phosphate-phosphite separation, the fraction of the total activity found as pentavalent phosphorus is about 5% less than in Table 1. This may be due to absorption of carrier-free ion species or to exchange processes, but it does not detract from our general conclusions.

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References

1. LIBBY, W. F. *J. Am. Chem. Soc.*, **62**, 1930 (1940).
2. HULL, D. *Ibid.*, **63**, 1269 (1941).
3. WILSON, J. *Ibid.*, **60**, 2697 (1938).

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