pected relative innocuousness and therapeutic ineffectiveness of mercurial diuretics in patients with renal disease may be related to the presence of marked proteinuria.

Addendum. After submission of this paper, we became aware of work done by Havill, Lichty, and Whipple,(21) showing that the tolerance of dogs for mercury bichloride was increased by frequent hemoglobin injections. It seems reasonable to assume that the mechanism of protection from the toxic action of the metallic ion was similar in this instance, since hemoglobin, like albumin, is reabsorbed by the proximal tubule cells until saturation of the reabsorptive capacity occurs, although Havill and his co-workers did not relate the protective effect to the occurrence of hemoglobinuria, which they did not measure, suggesting instead that the pigmented moiety of the hemoglobin molecule was responsible for the protection.

21. Havill, W. H., Lichty, J. A., Jr., and Whipple, G. H., *J. Exp. Med.*, 1932, v55, 627.

Summary. Administration of human albumin to rats, with resultant proteinuria, prior to administration of mercurial diuretics, sharply reduces renal toxicity of the mercurial. This effect is attributed to inhibition of mercurial reabsorption when the tubules are saturated with protein.

Simultaneous administration of human albumin and the mercurial enhances toxicity. This effect is attributed to the more rapid absorption of mercurial, concomitant with more rapid reabsorption of protein during the tubular loading phase.

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Fetal Death and Maldevelopment Resulting from Maternal Vitamin A Deficiency in the Rat.* (17543)

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The course of pregnancy may be severely altered by feeding female rats a diet restricted in vitamin A, the severity of the alteration being roughly proportional to the degree of vitamin deprivation. Death and resorption of the fetuses, prolongation of pregnancy, and delivery of stillborn young were reported by Mason(1) and Cannon(2) who employed deficient diets with little or no supplement of vitamin A or carotene. Warkany and Schraffenberger(3,4) using similar diets supplemented with small amounts of carotene also noted a high incidence of fetal resorption; but, by interrupting pregnancy at various times from the 13th day to term, they were able to obtain a number of living and recently dead fetuses and newborns suitable for histologic study. The eyes of these offspring were found to bear several developmental anomalies, notably: folding and eversion of the retina, persistence of the choroidal fissure, absence of the ciliary body, and postlenticular fibroplasia. Thus, deficiency of vitamin A in the diet of female rats prior to and during pregnancy not only reduces fecundity by

* Supported by a grant from the Nutrition Foundation, Inc., New York.

1. Mason, K. E., *Am. J. Anat.*, 1935, v57, 303.

2. Cannon, M. D., PROC. SOC. EXP. BIOL. AND MED., 1940, v44, 129.

3. Warkany, J., and Schraffenberger, E., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 49.

4. Warkany, J., and Schraffenberger, E., *Arch. Ophthal.*, 1946, v35, 150.

causing fetal death and resorption, but also causes developmental anomalies in many of the fetuses that escape intrauterine death.

The widespread interest aroused by the success of Warkany and his collaborators in producing congenital malformations by means of maternal vitamin A deficiency makes it desirable that the work be repeated in another laboratory. To date the only published account of attempts to repeat the experiment elsewhere was that of Jackson and Kinsey,(5) who did obtain a few newborn rats with ocular malformations; but the positive results of this investigation were of such limited nature as to cast doubt on the facility and regularity with which such defective young might be produced. One of us (J.G.W.) has been associated with Dr. Warkany in a morphologic and embryologic study of the malformed fetuses and newborns obtained from the experiments carried out in Cincinnati. Since certain material needed for further investigation of this subject (placentae and early embryonic stages prepared especially for histochemical study) was not readily available from Dr. Warkany's laboratory, it was decided to produce it in this laboratory. The present report relates the repetition of the original experiment and, in addition, presents some observations not previously recorded.

Experimental. Vitamin A deficiency was induced and maintained by the same diets and experimental procedures as were employed in Warkany's original experiments.(3,4) except for the quantity and method of administration of the carotene supplement, and a few other matters of incidental nature specified below. Rats of two strains were used, the Albino Farms and the Wistar; the latter had not been used previously for this type of experiment. Since no difference in reaction to the experimental procedures was detected in animals from the different sources, the matter of strain will receive no further mention. A total of 36 young females were raised from weaning on a semipurified, "preparatory" diet which contained very little vitamin A or carotene. To insure continued growth to maturity a 15 microgram dose of carotene in olive oil was administered orally each week. Presumably this dosage did not permit appreciable storage of vitamin A. When the animals had attained a body weight of about 160 g, each female was given an opportunity to breed with a male of the same strain fed an adequate diet. If mating occurred, as indicated by presence of sperm in the vaginal smear, the female was transferred to a more highly purified diet which was adequate in all known respects except for the lack of vitamin A. No further supplemental dosing with carotene was given. The day on the morning of which sperm were found in the vaginal smear was considered the first day of pregnancy. Eight control females were fed the same diets and subjected to the same procedures but were given weekly supplements of 150 μg of carotene, both prior to and during pregnancy.

From the experience of other investigators,(1,2,6) it was expected that a large percentage of the pregnant females on vitamin A deficient diets would resorb all of their conceptuses at some time before term. On the other hand, Warkany and Roth(6) found that approximately 25% of the conceptuses which survived until the latter part of pregnancy were developmentally normal. The margin between fetal destruction and normal development, therefore, appears to be very narrow. One of the aims of the present experiment was to attempt to establish criteria by which one might predict ultimate destruction of the conceptuses, thereby making it possible to obtain prior to death embryos which were destined to undergo resorption later. Mason(1) observed that the presence of cornified cells in the vaginal smear during pregnancy was frequently associated with subsequent fetal death and resorption. Warkany and Schraffenberger(4) considered excessive amounts of blood in the vaginal smear and a loss of weight by the mother as indications that resorption had already begun. It was hoped that by carefully watching for all of these signs (cornified cells in the vaginal smear, excessive blood in the smear, and loss of weight

5. Jackson, B., and Kinsey, V. E., *Am. J. Ophthal.*, 1946, v29, 1236.

6. Warkany, J., and Roth, C. B., *J. Nutrition*, 1948, v35, 1.

TABLE I.
Course and Termination of Pregnancy in Vitamin A Deficient and Control Rats.

Age of fetuses when removed or delivered, days	Days of pregnancy on which untoward signs were observed			Condition of young		
	Cornified cells in smear (25% or more)	Abnormal blood in smear	Wt loss by mother	No. dead or resorb.	No. living	Malformed
22	—	—	19	0	11	No
22	—	—	—	2	7	Yes
22	—	—	11	2	8	Yes
20	6–16, 18, 20	18, 20	14	all		
19	7–14, 17, 18	10, 11, 16, 19	—	3	6	Yes
19	—	19	14	4	8	Yes
19	19	15, 17, 18	15, 18, 19	all		
19	7–18	10, 11, 19	15, 16	all		
18	4–11, 13, 14, 17, 18	15, 17, 18	13	all		
17	—	15, 16, 17	12, 13, 15	7	5	Yes
16	13–16	15	12, 14	all		
16	8, 10, 11	11, 15	14, 15, 16	all		
15	8–15	15	—	all		
15	1–13	11	14, 15	all		
15	9–11, 13	15	11	all		
15	4, 9, 14	15	13, 15	5	5	Yes
Controls						
22	—	—	12	3	9	No
22	—	15, 16	—	0	3	No
22	13, 14	17–20	13	4	4	No
22	—	—	—	0	14	No
22	—	—	—	0	7	No
20	—	—	—	1	13	No
17	—	—	13	0	11	No

by the mother) the future course of pregnancy might be determined with some degree of accuracy.

Fetal death. Of the 36 females raised until maturity on the preparatory diet 34 mated as indicated by sperm in the vaginal smear, but only 30 were later verified to have become pregnant. The first 16 pregnancies, after being transferred to the more highly purified diet, were allowed to continue to term or until there was definite evidence of prior resorption of some of the fetuses. Thus, this group provided a check on the reliability of the proposed criteria to be used in predicting intra-uterine destruction of the fetuses. Only 3 of these 16 pregnancies were sufficiently uneventful that they were allowed to proceed to the 22nd day of gestation (Table I). Thirteen were interrupted earlier by killing the mother when it was judged unlikely that any of the conceptuses would survive until term. Such judgment was made after at least two, and usually all, of the following signs became positive: 1) 25% or more of cornified cells in the vaginal smear during pregnancy; 2) blood in the vaginal smear in excess of, or at times other than, that characteristic of the "placental sign", which appears normally as a trace between the 11th and 15th days; and 3) a loss of weight by the mother, particularly after the 10th day.

It is apparent from the data in Table I that these criteria were of value in indicating the fate of the fetuses. In the absence of cornified cells and abnormal quantities of blood in the vaginal smear, a majority of the fetuses may be expected to survive until the 22nd day, as was observed in 3 vitamin A deficient and a large percentage of the control mothers. Conversely, cornified cells, to the extent of 25% or more were observed at one time or another in 11 of 13 females whose pregnancy was later interrupted. All fetuses in 9 and several in the remaining 2 pregnancies were dead or already undergoing resorption. Two A deficient females, whose pregnancy was interrupted for other reasons, failed to show as much as 25% of cornified

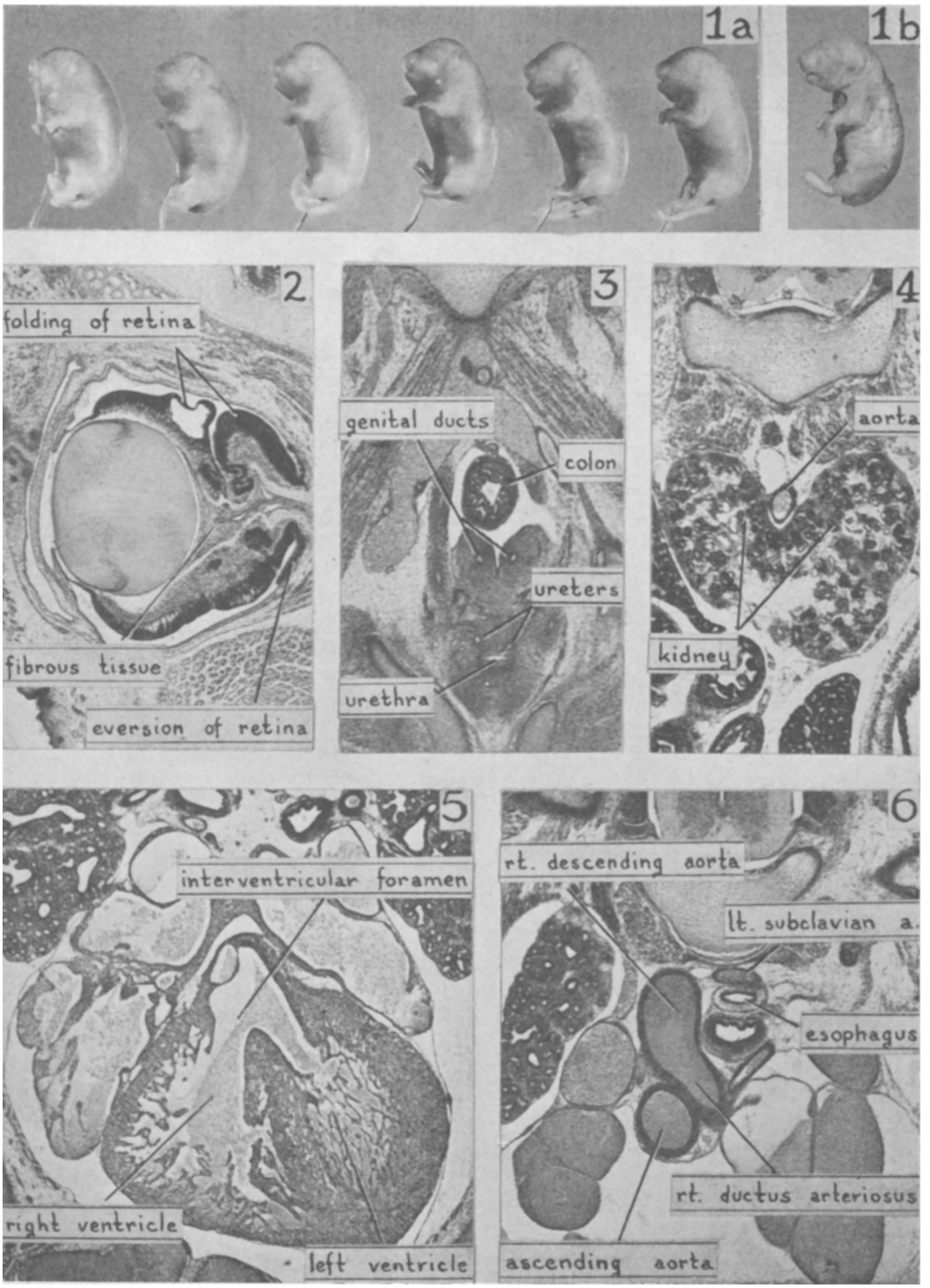

FIG. 1. a. Varying degrees of edema in young rats removed on the 22nd day of gestation from a mother fed vitamin A deficient diets. b. A normal newborn rat from a mother fed vitamin A deficient diets but supplemented with 150 μg of carotene per week.

FIG. 2. Abnormal eye of a 22-day rat fetus, from a vitamin A deficient mother. The nervous layer of the retina is deeply folded, contains cysts, and is everted on to the pigmented layer in the region of the optic stalk and along the persistent choroid fissure. The vitreous chamber is reduced to a small cone-shaped volume which is filled with fibrous connective tissue and remnants of the hyaloid artery. Retinal coloboma was present but is not apparent in this section. × 25.

FIG. 3. Ectopic openings of ureters in a 19-day rat fetus from an A deficient mother. The left ureter is seen to terminate in the urethra, at some distance below its usual termination at the base of the bladder (normally attained by the 17th day). The right ureter emptied at an even lower level in the urethra. × 25.

FIG. 4. Horseshoe kidney in a 19-day rat fetus from an A deficient mother. × 25.

FIG. 5. Persisting interventricular foramen in the heart of a 19-day rat fetus from an A deficient mother. The foramen is normally closed by the 17th day of gestation. × 25.

FIG. 6. Right-sided aorta and right-sided ductus arteriosus in a 19-day rat fetus from an A deficient mother. The left subclavian artery is also anomalous in that it arises from the descending aorta and passes behind the esophagus to reach the left shoulder. × 25.

cells at any time during pregnancy; and both yielded some living young.

Blood in the vaginal smear, in excess of or at times other than that seen during the placental sign, was noted in all of the pregnancies which were later interrupted (Table I). This would seem to be in itself an excellent indicator of fetal destruction, were it not for the fact that a subjective estimate of the quantity of blood is necessary when it appears at the same time as the placental sign. A further limitation of this criterion was noted in that, prior to the 15th day, death and partial resorption of all fetuses often occurred before excessive blood was present in the vagina. On the other hand, when resorption of some conceptuses began during the last third of pregnancy, the quantity of abnormal blood was roughly proportional to the extent of fetal destruction. Two controls showed what was judged to be abnormal blood, and in both cases there was evidence that resorption of some fetuses had begun late in pregnancy.

Loss of weight in the mother was recorded for all but 2 of the pregnancies interrupted prematurely. Also, it was observed in 3 of the controls. The degree of loss was of more significance than the mere fact that a female failed to gain weight. Both the degree and rate of loss appeared to be determined by the number of fetuses undergoing resorption at any one time, as well as by the size of the fetuses at the time of their death.

Thus, of the 3 criteria used in following the course of pregnancy in vitamin A deficient females, no one was found to entirely reliable as an indicator of fetal destruction. However, when all 3 of these signs became positive during pregnancy, several if not all of the fetuses of that pregnancy were later found to have undergone intra-uterine destruction. Pregnancies interrupted after the appearance of only 2 of the criteria, despite the fact that they were not interrupted immediately, generally yielded some living young.

In an additional group of 14 vitamin A deficient females (not included in Table I) pregnancy was interrupted immediately when the second of any 2 of the signs described above became positive. Accordingly, all of these were killed between the 11th and 15th days. Of this group, 12 females yielded intact embryos, and all but one of these contained more intact than resorbed embryos. This procedure, therefore, proved successful as a means of recognizing impending destruction of the conceptuses, thereby providing embryos and placentae which, although doubtless affected by maternal vitamin A deficiency, had not yet begun disintegration. This material is being prepared for histologic and histo-chemical study of the early stages of abnormality in the embryos and of possible associated changes in the placentae.

Malformation of the surviving fetuses. Nineteen of the vitamin A deficient females mentioned in the preceding section yielded offspring suitable for histologic study, but only the fetuses of 15 days gestational age or older have yet been examined for malformations. Of the 7 "litters" comprising the latter group (Table I), all but one contained malformed fetuses. The one litter of normal offspring was from one of the 3 pregnancies which proceeded to term without appreciable indica-

tions of fetal destruction. No malformations or other abnormalities were found in the offspring of the control animals, which were fed the same diets and subjected to the same procedures as the A deficient mothers except that the controls were given 150 micrograms of carotene per week, both prior to and during pregnancy.

From the 6 abnormal litters older than the 14th day, at least 2 and in most cases all of the young have been serially sectioned and studied. Abnormality of the older fetuses was evident before sectioning by external appearance alone: the characteristic edema of head, neck and thorax was apparent to a variable degree in all (Fig. 1 a and b). Hemorrhage in the region of the eye and prenatal opening of the lids were occasionally noted.

Warkany and collaborators(7) have reported that ocular abnormality was the most prevalent type of developmental defect resulting from material vitamin A deficiency. This was substantiated by microscopic study of animals from the 6 abnormal litters listed in Table I. All such animals examined had ocular anomalies, the more common of which were: folding and eversion of the retina, presence of a mass of fibrous connective tissue in the much reduced vitreous chamber, persistence of the choroidal fissure (coloboma), and absence of the ciliary body (Fig. 2).

Genito-urinary organs also were frequently found to be the sites of malformation in earlier observations.(8) The same types of defects have again been encountered in a similar percentage of animals. Among the commoner abnormalities in both instances were hypoplasia of renal parenchyma, renal ectopia, ectopic ureteric openings (Fig. 3), incomplete development of Müllerian ducts, abnormal retention of heterologous genital ducts in both sexes, aplasia of vagina and faulty differentiation of the urogenital sinus. Less frequent abnormalities were horseshoe kidneys (Fig. 4), cryptorchidism, hypospadias, and atresia of genital ducts and ureters.

Cardiovascular anomalies were found in 44% of a selected group of fetuses and newborns from vitamin A deficient mothers in a previous study.(9) In the present, somewhat smaller group of animals an even higher incidence (more than 60%) of malformations of the heart and major arteries was observed. In general, the anomalies were of the same type, but a few varieties not previously seen were encountered. Failure of the interventricular septum to close was again the most common defect of the heart (Fig. 5). Variations in the aortic-arch pattern frequently observed in both instances were: distally-arising right subclavian artery, right-sided arch of aorta (Fig. 6), double arch of aorta, absence of 4th arch, absence or bilateral persistence of the ductus arteriosus, and absence of a pulmonary artery.

The occurrence of keratinizing metaplasia in epithelia of the lower genito-urinary tract of the offspring from A deficient mothers has been interpreted as indicating that such offspring had themselves experienced *in utero* a state of deficiency similar to that in the mother.(10) The same interpretation may be applied to the present group of animals, in which metaplastic keratinization was again observed in the genito-urinary tract of fetuses older than 18 days gestational age.

Summary. This report constitutes a confirmation of the original experiments of Warkany and co-workers in which congenital malformations were induced in fetal and newborn rats by maintaining the mothers on vitamin A deficient diets. In addition, a method has been standardized which permits prior recognition of imminent death and resorption of the conceptuses, thereby making it possible to collect many severely malformed fetuses that would otherwise be destroyed *in utero*. Three criteria that proved reliable in anticipating prenatal destruction of fetuses were: 1) the presence of more than 25% of cornified cells in the vaginal smear at any time during pregnancy; 2) the presence of blood in the vaginal smear in excess of or at times other than that

7. Warkany, J., Roth, C. B., and Wilson, J. G., *Pediatrics*, 1948, v1, 462.

8. Wilson, J. G., and Warkany, J., *Am. J. Anat.*, 1948, v83, 357.

9. Wilson, J. G., and Warkany, J., *Am. J. Anat.*, 1949, v85, 113.

10. Wilson, J. G., and Warkany, J., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 419.

comprising the usual placental sign; and 3) a loss of weight by the mother after the 10th day of pregnancy.

Malformations involving the eye, cardiovascular system, and genito-urinary tract were found in the fetuses or newborns from all but one of the pregnancies from which intact offspring were obtained after the 14th day of gestation.

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Beef Erythrocyte Extracts Reacting with Hemagglutinins in Infectious Mononucleosis and in Horse Serum Sickness. (17544)

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In previous publications(1,2) it was demonstrated that 2 serologically active substances can be isolated from the stroma of beef erythrocytes; one reacts with the hemagglutinins that are formed in infectious mononucleosis, the other, with those formed in horse serum sickness. The stroma of beef erythrocytes was first exhaustively extracted with acetone and then with 100% ethanol at room temperature, thus removing serologically inactive substances to a large extent. In order to isolate the specific reactants the stroma residue was then extracted with boiling 100% ethanol, and subsequently, with boiling 80% ethanol. As can be seen from Table I, the 80% ethanol extract yields the so-called mononucleosis antigen (M.A.) and the extract obtained with boiling 100% ethanol, the so-called serum sickness antigen (S.S.A.). Complete and distinct separation of the two serologically active substances could not be accomplished. The fractions do not correspond to pure haptens. However, on the basis of serological activity it could be assumed that the individual fractions were only 2-6% contaminated by the heterogenic fractions. Since the individual fractions are serologically highly active it was thought probable that they might demonstrate significant chemical differences which might point to the nature of the individual haptens. On the basis of previous investigations the nature of these haptens was disclosed only to the extent that they were found to be thermostable, not digestible by trypsin and pepsin, and did not give reactions characteristic for proteins. This report deals with the chemical analysis of the serologically active fractions of the beef erythrocytes. In addition, the glucose and glucosamine content after hydrolysis is presented.

Methods. 1. The C–, H– and N– determinations were done according to Pregl's method of quantitative elementary microanalysis.

2. Phosphorus was determined as ammoniumphosphomolybdate.

3. The reducing power was determined according to the Hagedorn-Jensen method and calculated for the glucose equivalent. The fractions were hydrolyzed at 100°C with 2 N HCl for 2 hours, then neutralized.

4. The glucosamine determination was done according to the method of Boyer and Fuerth as modified by Brunius.(3) 25-75 mg of dry substance were suspended in 7.5 ml of 4 N HCl and diluted with distilled water to 10 ml. This was hydrolyzed at 100°C in a sealed test tube for 5 hours. After filtration, whenever necessary, color clarification was effected by charcoal adsorption. This solution was carried to dryness over KOH and redissolved in 2 ml of distilled water. This, after

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1. Schwarzweiss, H., and Tomcsik, J., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 558.

2. Tomcsik, J., and Schwarzweiss, H., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 562.

3. Brunius, F. E., *Chemical Studies on the True Forssman Hapten, etc.*, A. B. Fahlcrantz Boktryckeri, Stockholm, 1936, p. 141-144.