from hemosiderin in 25 days. The results indicate that iron may be present in more than one form in hemosiderin. Iron that is readily dissolved during the first 24 hours undoubtedly represents a compound(s) which differs in composition from a relatively insoluble iron-containing residual material. To explain the gradual increase in iron after first day of extraction, it may be assumed that iron is firmly bound to an insoluble protein which undergoes structural changes during extraction or that an insoluble iron complex is gradually converted to a hydrate(6). The relatively insoluble iron residue is probably not identical with limonite(2) since this material is insoluble in Na_2EDTA.

In 6 samples of water-soluble fraction of iron-free hemosiderin, the apoferritin nitrogen concentration constituted 1.2 to 3.5% of total hemosiderin nitrogen. These results represent .5 to 1.5% of ferritin in hemosiderin. These results may not be quantitative since the most reliable data would be obtained by immunological technics. If it is assumed that all ferritin iron reacts with Na_2EDTA during the first 24 hours, it represents only a small portion of extractable iron.

Summary. 1. It has been demonstrated that iron is extracted from horse spleen hemosiderin at different rates by disodium ethylenediamine tetraacetate. During the first 24 hours of extraction, between 21 and 70% of total hemosiderin iron was removed by chelating agent from 8 hemosiderin preparations which contained between 33.5 and 19% iron, respectively. The amount of iron removed is inversely proportional to total hemosiderin iron concentration. Subsequently, iron is dissolved at a slow rate until practically all is removed during 25 days extraction. 2. Analytical data for amount of ferritin present in hemosiderin are presented.

1. Ludewig, S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 514.
2. Schwietzer, C. H., Acta Haemat., 1953, v10, 174.
3. Greenberg, D. M., 19th Ross Pediat. Research Conf., Portland, Ore., Oct. 20-21, 1955.
4. Granick, S., Michaelis, L., *J. Biol. Chem.*, 1943, v147, 91.
5. Laurell, C. B., *Acta Physiol. Scand.*, 1947, v14, Suppl. 46.
6. Schwietzer, C. H., *Deutsche med. Wschr.*, 1952, v77, 17.

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Strain Differences in Mercury Retention by Chicks.* (24607)

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Extensive information on pharmacology and toxicology of mercury is available(1,2). However, in a study of metabolism of organic mercury compounds, a difference in retention of mercury in strains of chicks was noted. This difference in strains of chickens selected for susceptibility and resistance to lymphomatosis is reported here. No reports have been found indicating a relationship between mercury retention and resistance or susceptibility to lymphomatosis. Pending further study, we do not imply such a relationship exists. No information is available on m.l.d. of phenylmercury acetate (PMA) with chicks. Löliger (3) fed a fungicide containing 5.1% phenylmercury pyrocatechol to hens at 1 g (26.5 mg mercury)/kg body weight without deaths. Hagen(4) reported the dose of same phenylmercury compound injected intravenously causing death to mice was 50-100 mg (25-50 mg mercury)/kg body weight. Basic phenylmercury nitrate injected intravenously at 8.6 mg mercury/kg body weight was fatal to rabbits(5). At Western Washington Exp. Station (WWES), intramuscular injections of 9 mg mercury at PMA/kg body weight caused

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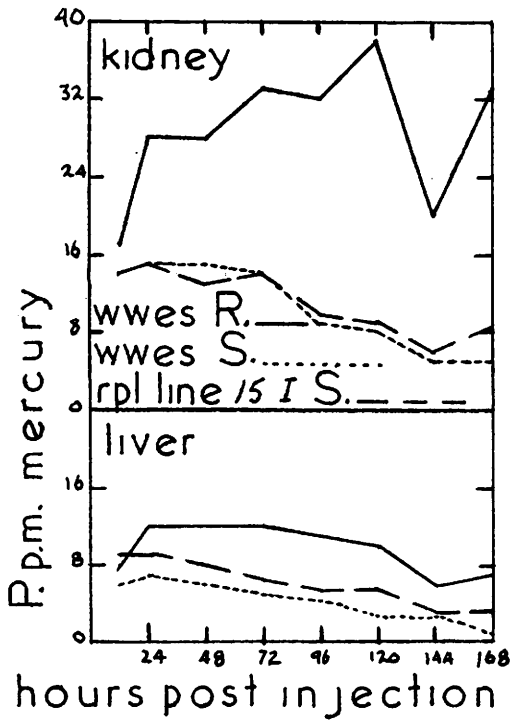

FIG. 1. Retention of mercury in livers and kidneys of resistant and susceptible birds following inj. of phenylmercury acetate.

no deaths in 20 chicks. Similarly per os. 18 mg mercury/kg caused 4 deaths in 23 chicks.

Methods. Chicks in Exp. 1 were 3 strains of White Leghorns. Two of these strains were selected from a common ancestry for resistance and susceptibility to visceral, neural, and ocular lymphomatosis incurred by contact exposure at WWES for 26 generations. Lymphomatosis mortality in the WWES Resistant (R) and Susceptible (S) lines resulting from contact exposure was very different for many generations, although recent generations have shown little difference. The third strain was Line 15 I of the U.S.D.A. Regional Poultry Research Lab. (RPL) East Lansing, Mich.,† a closed flock for many years, the offspring are highly susceptible to RPL-12 strain of lymphomatosis. In Exp. 2 only the WWES lines were used. *Exp. 1.* Male chicks were fed a practical type diet *ad lib.* from time of hatch. One hundred and thirty chicks, weighing 175 to 600 g. were injected intramuscularly in the breast with a solution of 3 mg/ml of recrystallized PMA in isotonic phosphate buffer at pH 7.2 at 3 mg mercury/kg of body weight. At intervals between 12 and 168 hours after injection, the chicks were decapitated and liver and kidneys excised and analyzed for total mercury(6).

Results. Results of mercury analyses are shown in Fig. 1. Concentration of mercury in liver tissue of the WWES R birds is greater than in the 2 S strains (WWES S and RPL 15 I). Analytical data for kidneys show an even greater difference between R strain and 2 S strains than do the data for livers.

Percentage of original dose found in liver plus kidneys for the same time was calculated. At 12 hours after injection, the average retention by all lines was 15 to 18% of original dose. With S lines, after temporary slight increases, the percentage retention decreased to 7% or less at 168 hours. R line retention increased to 34% at 72 hours, plateaued until about 120 hours, and declined to 23% at 168 hours post injection.

Analysis of variance of data from 96 birds that could be treated statistically, showed that concentration of mercury in livers was highly significant ($P < .01$) for time after injection and between strains. Similarly with data from kidneys, the differences for time after injection, between strains and interaction between time and strains were highly significant ($P < .01$).

Exp. 2 investigated retention of several types of compounds of mercury. The materials used were (1) PMA as in Exp. 1; (2) mercuric chloride in isotonic saline, with pH adjusted to 7.2 with isotonic sodium bicarbonate; (3) recrystallized ethylmercury chloride dissolved in isotonic saline; (4) sodium O-[(3 hydroxymercuric - 2 - methoxypropyl)-carbamyl] phenoxyacetate (Salyrgan theophylline Winthrop) diluted with sterile saline and standardized before injection. Salyrgan was selected because of reported very low retention of mercury by the animals(7). All mercurials were injected intramuscularly into the breast of WWES S and R birds at 3 mg mercury/kg body weight. Males weighing

† The authors gratefully acknowledge cooperation of Dr. B. R. Burmester in furnishing hatching eggs of this strain.

TABLE I. Percentage of Injected Dose of Mercury Compounds Recovered in Liver and Kidneys of Chickens.

Compound	Strain bird	% injected mercury recovered 24 hr L*	24 hr K*	96 hr L	96 hr K	168 hr L	168 hr K
ØHgOAc	R	15.5	13.3	17.8	17.7	13.2	13.8
	S	9.8	8.7	4.8	5.2	4.6	4.7
$HgCl_2$	R	6.0	13.8	9.0	20.5	9.9	22.1
	S	4.9	6.7	3.8	6.7	4.3	5.8
EtHgCl	R	14.1	3.0	23.6	6.5	23.5	7.0
	S	11.1	2.8	19.5	4.1	17.8	4.1
Salyrgan	R	2.9	1.8	1.9	1.7	3.2	1.6
	S	1.0	.5	1.1	.8	2.9	.6

* L = Liver, K = Kidney.

166 to 285 g were used. The 92 birds involved were fed as in Exp. 1. After 24, 96, and 168 hours post injection, the birds were decapitated and liver and kidneys removed and analyzed for mercury.

The results in Table I indicate that chickens of the R strain retained more of the mercury compounds than did birds of the S strain. Mercuric chloride and PMA are retained in about the same 3:1 ratio by the 2 strains. With PMA the retention is about equal in liver and kidneys, whereas in birds injected with mercuric chloride, kidneys retained twice as much mercury as the livers. These data for liver and kidney retention with these 2 mercurials are in agreement with findings of Prickett *et al.* using rats(8). With ethylmercury chloride injections, smaller differences in retention were observed between livers of R and S birds. In contrast to preceding compounds, mercury was retained predominantly in the liver. Salyrgan mercury was retained only slightly by either line, athough, as with the other mercurials, the greater retention was in the R line.

From our data, phenylmercury and inorganic mercury appear to be metabolized in a similar manner. Phenylmercury appears to be broken down rapidly and thereafter is metabolized like inorganic mercury. Ethylmercury appears to remain intact longer in the animal body as suggested by Swensson (9). Salyrgan is excreted without decomposition to inorganic mercury(10). Our findings are in agreement with these reports.

While variation was observed in our experiments, except for post injection times of 24 hours or less, only on 2 individual observations was the lowest percentage retention in the R line less than the highest of either of the S lines in both experiments. Rate of absorption from injection site and establishment of equilibrium within the bird may account for short time variabilities. Strain differences in experimental animals may account for variability of other materials as well as mercurials.

Summary. Studies made with 3 strains of chicks showed one strain to be significantly different in retention of mercury in liver and kidneys, following intramuscular injections of phenylmercury acetate or mercuric chloride. Differences following injection of equivalent amounts of mercury as ethylmercury chloride or Salyrgan were much less. Mercury retention was greater in the strain selected for resistance to lymphomatosis than in the 2 susceptible strains which showed little difference.

1. Veress, H. E., Smelcer, N. K., *Mercury Toxicity,* USAEC Unclassified Document TID-3067, May 1957. Oak Ridge, Tenn.

2. Mercury and Its Compounds. *Ann. N. Y. Acad. Sci.,* 1957, v65, 357-652.

3. Löliger, H. C., *Z. angew. Zool.,* 1955, 453.

4. Hagen, U., *Arch. exp. Path. u. Pharm.,* 1955, v224, 193.

5. Council on Pharmacy and Chemistry, *Am. Med. Assn. J.A.M.A.,* 1941, v117, 1984.

6. Polley, D., Miller, V. L., *Anal. Chem.,* 1955, v27, 1162.

7. Kessler, R. H., Lozano, R., Pitts, R. F., *J. Clin. Invest.,* 1957, v36, 656.

8. Prickett, C. S., Laug, E. P., Kunze, F. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 585.

9. Swensson, A., *Acta Med. Scand.,* 1952, v143, 365.

10. Leuschner, F., *Arch. exp. Path. u. Pharm.,* 1957, v230, 513.

Received November 14, 1958. P.S.E.B.M., 1959, v100.