AN INVESTIGATION OF BLOOD LIPID LEVELS IN ESTROGEN TREATED COCKERELS

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Preface

As early as 1916, Lawrence and Riddle (1) noted that in laying hens, the level of plasma lipids was markedly increased over that of the immature female bird. Later (4) workers at the University of California reported similar findings on the fatty acid, cholesterol, and phospholipid levels, when estrogenically potent substances present in pregnant mare urine were administered to young pullets and cockerels. With the advent of the synthetic estrogens and more specifically the highly potent diethyl stilbestrol, various workers found that these substances gave even more dramatic results.

Little or nothing has been shown to definitely establish the primary origin of the fat in the lipemic blood. It seems likely that there are two possible sources from which the large amounts of fat may come: a rapid release of depot fat and its mobilization as circulating plasma lipid, or the diversion of ingested feed from the normal pathways of metabolism.

The results of a series of experiments designed to indicate which of these two mechanisms might be functioning are reported here.

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Introduction

Hormonal control of plasma lipids was suggested by Lawrence and Riddle (1) who observed that at the onset of laying in hens there was a marked increase in blood fat. These reports were confirmed and extended by Warner and Edmond (2), Riddle and Burns (3), the latter workers using pigeons. A marked rise in blood fat levels following administration of ovarian hormone preparations was shown by Entenman, Lorenz and Chaikoff (4) in the domestic fowl. These workers administered, over a 27 day period, from 1000 to 2000 rat units of estrogenic substances obtained from pregnant mare urine. They noted that total plasma lipids increased from 370 mg. per cent to 1530 mg. per cent.

The unusual estrogenic potency of diethyl stilbestrol (4,4'-Dihydroxy-α,β-diethyl stilbene) was recognized by Dodds and his coworkers (5) soon after the compound had been synthesized by them. Dodds et al., however, had assayed the effect of their new compound using ovariectomized female rats, and it remained for Zondek and Marx (6) to demonstrate the activity of this substance in the fowl. They reported that a total dosage of 24 mg. given over a 6 day period caused the blood fat in chickens to rise from 125 mg. per cent to 5438 mg. per cent. This established the synthetic hormone as highly potent in chickens.

Flock and Bollman (7) similarly reported dramatic changes in all fatty constituents of the blood when diethyl stilbestrol was injected in a solution of peanut oil. Lorenz (8) observed

general fattening in chickens, accompanied by an extreme lipemia when pellet intramuscular implants of diethyl stilbestrol were used. While it had been shown that oral administration of this substance was relatively without effect. Thayer, Jaap and Penquite (9) found the dimethyl ether of diethyl stilbestrol to be highly potent when given orally.

The actual mechanism by which the pronounced lipemia is produced is not known. Riddle, Senum and Rauch (10) have reported that in pigeons and doves, pituitary gonadotrophin or the follicle-stimulating (FSH) factor stimulates the production of estrogens by normal ovarian tissue and the estrogens in turn produce what is termed a physiological lipemia related to reproduction. Injected estrogens on the other hand, Riddle et al. report, exert the same action in the presence or absence of the gonads (testes), adrenals, both lobes of the pituitary, pancreas, and the parathyroids. Riddle and coworkers have shown increases in blood fat for birds treated only with estrone and estradiol benzoate in the absence of each of the above mentioned glands. They have not used diethyl stilbestrol in the same manner. It seems likely, however that the synthetic estrogen acts in a similar fashion, and that both synthetic and natural substances may possibly be functioning somewhat independent of the pituitary.

The origin of the excess blood fat produced by estrogenic substances has never been adequately demonstrated. Flock and Bollman (7) have suggested that a mobilization of depot fat is responsible but have submitted no evidence to support this view. Bird (11) attempted to show that increased fat would be

absorbed from the gastro-intestinal tract when a bird was under the influence of estrogenic stimulation. He found no significant change in the fecal fat and it is therefore concluded that the usually efficient absorption of fatty materials thru the intestinal mucosa is unimproved by estrogens.

In this investigation both sources of the blood lipids mentioned above were considered possible, namely an estrogenically stimulated mobilization of the depot fat or retention in the blood stream of fat available from the diet, perhaps augmented by conversion of dietary carbohydrate to fat. Both of these mechanisms might indeed be functioning simultaneously.

Preliminary studies during the summer of 1948 had given indication that birds having little or no body fat stores did not develop a lipemia upon estrogen administration.

EXPERIMENTAL

Phase I (a)

In November of 1948, 24 male New Hampshires about eight weeks old were selected from the flock on the Poultry Farm of the Oklahoma A. & M. College. The birds were divided into four groups: two consisted of six birds each, which were held to a restricted ration, one of the two being treated with estrogen while the other was held as a control lot; the second two groups were full-fed, one being treated with estrogen and the other held as a control lot. The two dozen cockerels were confined indoors in regular laying cages about 30 inches square. In order to prevent cannibalism, the birds on a restricted ration were placed in individual cages while the full-fed birds were placed three per cage.

In restricting one group of birds as to feed intake, the following is the basis for the system employed: Lippincott and Card (12) report that the energy requirements for maintenance are a function of the size, age, sex, environmental temperature, and the degree of activity of the bird. The size and age are of greatest importance and these two only were considered. At about six weeks the growing bird requires about 1500 calories per square meter of surface area per day. Since the determination of the surface area of a live bird was impractical, the method of Mitchell (13) was used to compute it from the body weight. He gives the mathematical expression $S = 8.19\ W^{0.705}$ where S is the surface area in square meters and W is the weight of the

bird in grams. After trial it was found that 950 calories per day per square meter of surface area just failed to maintain the body weight of these experimental birds. Each bird on the restricted ration was weighed every other day and the calculated amount of feed corresponding to the weight of the bird given for two consecutive days. During a period of ten days, the twelve birds on a restricted intake lost on half to two thirds of their original body weight. During the same period the full-fed birds gained 0.5 to 0.8 pounds.

When this point had been reached six of the starved birds and six of the full-fed birds were injected with 2 cc. of soybean oil containing 50 mg. of crystalline diethyl stilbestrol* per ml. The remaining twelve birds were injected with 2 cc. of soybean oil. The injections were subcutaneously into the dorsal portion of the neck.

Just prior to the time of the injections of the estrogen, 2 cc. of blood were drawn from each bird by hypodermic heart puncture. At 12,24, and 48 hours following injection, blood samples were taken in the same manner. The blood was transferred from the syringe directly into 50 ml. centrifuge tubes calibrated to 40 ml. containing about 30 ml. of a mixture of 3 parts 95 per cent alcohol and 1 part diethyl ether.

The method of analysis of the blood for fatty acids and cholesterol was essentially that of Street (14). Certain modifications were made to make the method conform to the available equipment, and to increase the number of samples

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* The diethyl stilbestrol was provided through the courtesy of George A. Breon and Co.

that could be processed. A detailed description of the procedure follows:

The stoppered centrifuge tubes containing the alcohol-ether mixture, into which the blood had been transferred, were placed in a water bath at a temperature of about 60° C. and kept there with intermittent agitation for 15 to 20 minutes. They were then cooled and made to 40 ml. with the alcohol-ether mixture. After thorough mixing, they were centrifuged until a thorough separation was brought about between the extract and the whole blood. By means of a pipette 25 ml. of the supernatant were transferred to 50 ml. Erlenmeyer flasks, to which were added 1 ml. of saturated sodium hydroxide. The flasks were then placed upon a hot plate and the fat was saponified. When the alcohol-ether solvent was evaporated and only a pasty mixture of soaps and unsaponified material remained in the flasks, they were removed from the hot plate and allowed to cool. From a dispensing burette, 4 ml. of 9 N sulfuric acid were so added to the flasks that the soaps were washed off the sides. The heat of neutralization was sufficient to insure complete reaction of the acid with all of the soaps present and no external heat was applied. The flasks were allowed to cool to room temperature.

At this point, 15 ml. of specially purified (b.p. 30-35° C.) petroleum ether were added with agitation and the flasks stoppered. The petroleum ether was allowed to remain over the acidic solution for a period of several hours, sometimes overnight in order to bring about maximum extraction of the lipid materials. Bloor (15) assumes that the blood plasma lipids

after saponification, and hence that extracted by petroleum ether consists entirely of fatty acids and cholesterol. While this may not be strictly true, Street (14) suggests that no significant error is introduced in view of the knowledge of the composition of plasma lipids.

The ether layer was decanted carefully into 50 ml. centrifuge tubes, after which successively 7 and 5 ml. of the purified petroleum ether were added to the flasks, brought to boiling on a hot plate, and decanted into the first ether extract already in the centrifuge tubes. Centrifuging them separated out small portions of non-lipid materials which were unavoidably carried over in the decantation procedure. The ether extract was transferred to tared weighing bottles which had been brought to constant weight in a vacuum dessicator. The bottles were returned to the vacuum dessicator and the petroleum ether removed under reduced pressure by means of an aspirator pump, during a period of from 8 to 10 hours, usually over night.

The bottles now containing essentially only fatty acids and cholesterol, were weighed on an analytical balance; the increase in weight was expressed as total lipid.

In order to determine the value for the fatty acids, it is necessary to deduct the value for cholesterol present. The residue in the weighing bottles was taken up in 6 ml. of reagent chloroform and the cholesterol was determined on the solution by the method of Bloor (15) with one minor modification. For the sake of rapidity in adding the Liebermann-Burchard reagent to the samples, 4 ml. of concentrated sulfuric

acid were mixed with 80 ml. of chilled reagent acetic anhydride, and the resulting mixture was then added 2 ml. to each of the 24 samples and standards. The color development in the samples was compared in an Evelyn type photoelectric colorimeter with that developed in tubes containing known amounts of pure cholesterol dissolved in chloroform. The values of cholesterol were calculated as mg. per cent and subtracted from the total lipid values to give amounts of fatty acids in mg. percent.

The results of Phase I (a) are presented in Table 1.

Phase I (b)

This part of the experimental work was very similar to Phase I (a), the chief differences being: (1) no negative controls were used, the initial blood samples before injection being considered adequate; (2) the birds were restricted in their ration to a lesser degree with the result that the fat stores were not so completely dissipated as in Phase I (a); (3) blood samples were taken at 24 hour intervals over a longer period of time.

Phase I (b) was conducted during the summer of 1949 using 24, 12 week old male Barred Plymouth Rocks from the flock of the Poultry Department. The cockerels were confined as before and fed in an identical manner. Inasmuch as the older birds did not have as high a caloric maintenance requirement, the restricted ones did not lose as much weight. During eight days, however, they lost from 0.1 to 0.5 pounds while the full-fed birds were gaining in weight from 0.5 to 0.8 pounds.

Blood was taken from all 24 birds and they were immediately injected with 100 mg. of crystalline diethyl stilbestrol dissolved in 2 ml. of soybean oil. At intervals of 24 hours thereafter, 2 cc. of blood were drawn by heart puncture and treated in an identical fashion as in Phase I (a), analysis being made for fatty acids and cholesterol.

The results of Phase I (b) are presented in Table 2.

Phase II

An investigation into the relation of dietary levels of fat and the increases in blood fatty acids was undertaken in December of 1948. Twenty-four male New Hampshires of about 8 weeks from the Poultry Department were confined, one bird per cage, in the manner previously described. The birds were arranged in triads, the three being as nearly the same weight as possible. Each of the birds in a triad received one of the three rations shown in Table 3. The caloric intake of all three was limited to the minimum amount of feed, in terms of calories, consumed by any bird during the previous day. When the triads of birds had been equilibrated as to feed intake, 2 cc. of blood were taken from each by heart puncture. Twelve birds, four on each ration, were then injected with 100 mg. of crystalline diethyl stilbestrol dissolved in 2 cc. of soybean oil. The twelve remaining birds, again four on each ration, were injected with 2 cc. of soybean oil and thus held as controls. Blood samples were subsequently taken at 12, 24, and 48 hours after the time of injection. The blood was treated in precisely the same fashion

as in the two other experiments, being analyzed for total lipid and cholesterol, and the fatty acids calculated by difference.

The results of Phase II appear in Table 4.

In order to determine to what extent the differences in treatment, both dietary and the injection of diethyl stilbestrol, might affect the tissue lipid concentration, total tissue lipid was determined in a representative muscle group and in the liver. The left Pectoralis (superficial) muscle group and the entire liver were removed from two birds from each lot in Phase I (a) and four birds from each group in Phase I (b). The tissues were frozen and maintained in this state until analyzed. The tissues were dried at 65° C., chopped into small pieces and subjected to ether extraction. The total ether extractable material is designated as total lipid; the results of these analyses are presented in Table 5.

Table 1. The Effects of Diethyl Stilbestrol Injections On the Blood Lipids of Cockerels On Restricted and Full Ration.
Phase I (a)

Bird No.	Treatment: Amount of Ration	Treatment: Dosage of Estrogen	Fatty Acids: Before	Fatty Acids: 12 hrs. (Mg. percent)	Fatty Acids: 24 hrs. (Mg. percent)	Fatty Acids: 48 hrs. (Mg. percent)	Cholesterol: Before	Cholesterol: 12 hrs. (Mg. percent)	Cholesterol: 24 hrs. (Mg. percent)	Cholesterol: 48 hrs. (Mg. percent)
275	Restricted	100 mg.	479	495	379	375	113	97	109	265
276	"	"	386	492	305	548	110	116	231	115
277	"	"	360	439	441	---	144	217	311	---
278	"	"	594	639	669	809	166	209	259	255
279	"	"	351	499	556	1175	121	133	196	361
280	"	"	424	440	370	550	88	152	222	186
		Av.	427	501	453	691	Av. 124	138	221	236
281	Restricted	None	472	323	260	348	40	165	212	188
282	"	"	550	---	312	344	50	---	168	224
283	"	"	431	---	261	319	81	---	179	177
284	"	"	431	355	412	380	113	205	188	204
285	"	"	445	336	339	314	83	200	181	246
286	"	"	356	---	344	336	108	---	168	88
		Av.	448	338	321	340	Av. 79	190	183	188
287	Unrestricted	100 mg.	338	618	1278	3141	110	134	298	411
288	"	"	389	448	1202	2863	123	120	238	273
289	"	"	402	488	609	882	78	144	183	414
290	"	"	443	461	429	451	69	139	203	213
291	"	"	499	441	787	2507	37	135	333	333
292	"	"	393	551	1214	2695	47	153	226	361
		Av.	411	501	920	2090	Av. 76	138	247	334
293	Unrestricted	None	393	324	314	392	39	140	262	160
294	"	"	396	331	385	475	140	149	151	165
295	"	"	344	332	278	505	80	68	226	87
296	"	"	296	202	281	297	96	126	231	215
297	"	"	757	301	280	409	131	139	151	119
298	"	"	363	326	414	514	37	162	210	190
		Av.	425	303	325	432	Av. 87	131	249	156

Table 2. The Effects of Diethyl Stilbestrol Injections On the Blood Lipids Of Cockerels On Restricted and Full Rations. Phase I (b).

Bird No.	Amount of Ration	Dosage of Estrogen	Fatty Acids (Mg. percent) Before	24 hrs.	48 hrs.	72 hrs.	96 hrs.	Cholesterol (Mg. percent) Before	24 hrs.	48 hrs.	72 hrs.	96 hrs.
347	Restricted	100 mg.	534	667	1633	1953	794	138	85	207	207	150
348			626	1939	1939	2134	940	110	117	117	218	140
349			461	431	428	304	562	203	65	102	160	102
350			404	577	1270	749	436	76	119	130	91	132
351			748	793	1184	518	517	124	111	280	138	171
352			444	700	1997	2026	664	124	124	115	150	592
353			470	593	1837	3014	1428	162	127	107	98	244
354			414	613	986	329	384	82	107	102	111	104
355			550	1398	1000	1386	818	130	138	232	134	198
356			301	639	1690	882	579	283	113	134	326	221
357			496	363	946	1160	861	208	96	123	128	163
358			320	367	827	760	411	120	81	117	128	117
		Av.	481	590	1311	1185	763	Av.147	115	147	157	195
335	Unrestricted	100 mg.	484	1217	3227	1480	378	76	71	53	216	214
336			640	1315	3487	1338	180	80	69	313	198	292
337			538	1518	---	3256	989	78	138	127	192	171
338			423	1264	3577	3570	2301	153	96	62	318	163
339			449	1039	2196	2331	1359	183	73	148	381	305
340			425	1330	2880	3129	4228	95	142	272	903	260
341			288	1392	2457	875	298	192	120	295	165	150
342			450	1195	2377	1315	603	110	117	175	221	117
343			509	898	1457	453	284	155	102	207	123	100
344			778	1519	3402	2786	738	38	113	318	326	142
345			495	1397	3611	---	---	65	83	181	---	---
346			503	1221	---	3910	4344	65	123	146	829	128
		Av.	499	1275	2867	2005	1309	Av.108	104	191	352	176

Table 3. The Composition of Rations Used in Phase III

Ration Constituents	Percent Composition		
	Ration A	Ration B	Ration C
Corn	55	44.15	30.83
Soybean oil meal	35	30.92	25.89
Alfalfa leaf meal	5	5.56	8.17
$CaCO_3$	1	1.11	1.38
Bone meal	2	2.22	2.47
Fish solubles	2	2.22	2.47
Vegetable fat	-	8.90	19.73
Casein	-	4.89	11.22
Choline hydrochloride	(All rations contained 1 gm./lb. feed)		
Calculated percent fat	4.78	13.04	26.3
Caloric ratio	1.00	0.90	0.81

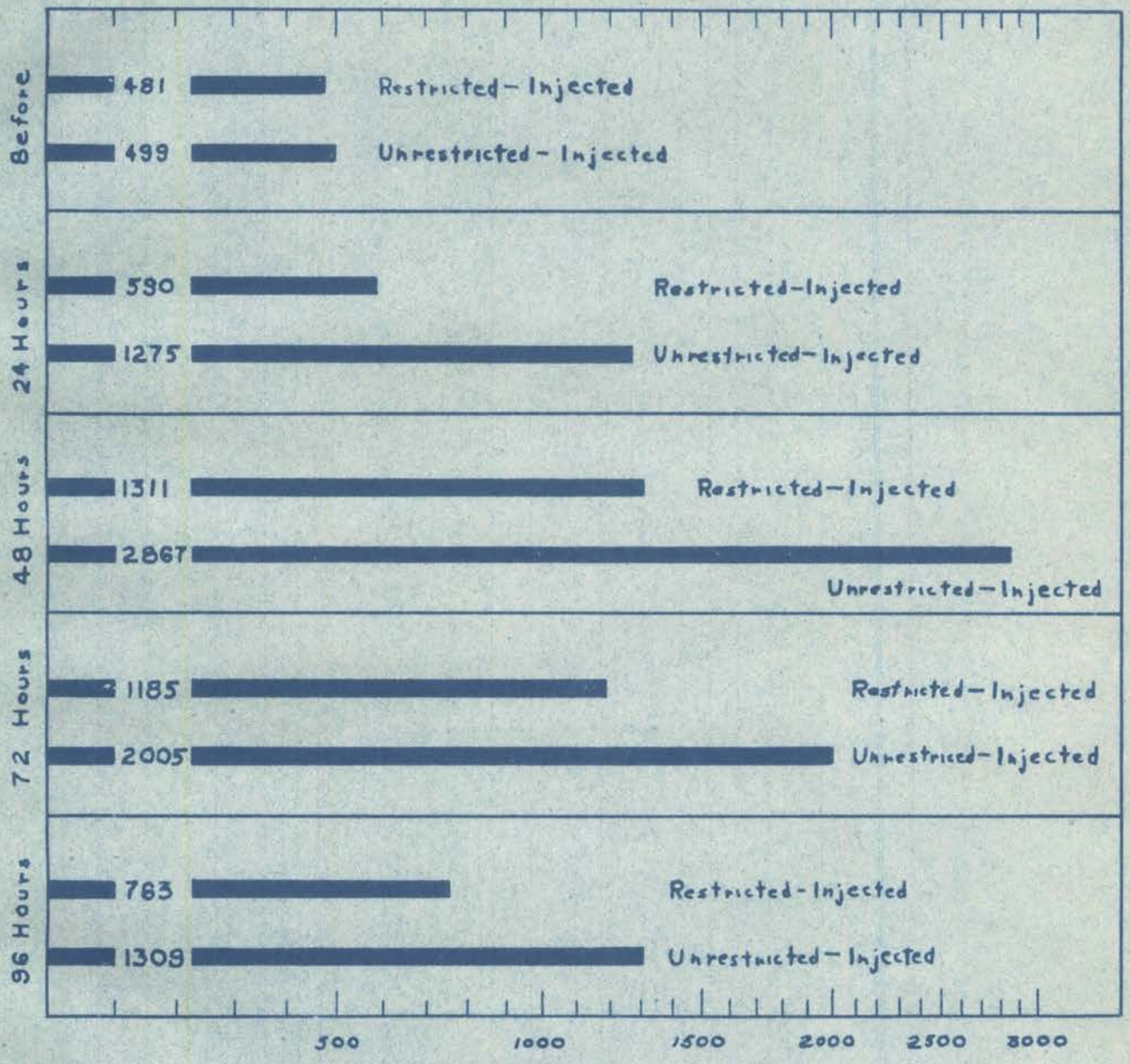
Table 4. The Effect of Diethyl Stilbestrol Injections On the Blood Lipids of Cockerels Fed Three Different Levels of Fat. (Phase II).

Bird No.	Treatment: Ration	Treatment: Dosage of Estrogen	Blood Fatty Acids (Mg. percent): Before	12 hrs.	24 hrs.	48 hrs.	Blood Cholesterol (Mg. percent): Before	12 hrs.	24 hrs.	48 hrs.
310	A	None	467	442	397	581	33	18	143	69
311	"		513	530	423	428	27	60	77	52
312			394	455	450	574	26	105	180	66
313			491	482	392	605	39	38	148	55
		Av.	466	477	416	547	Av. 31	55	120	61
314	B	None	581	542	---	558	39	58	---	62
315			400	492	437	453	10	38	93	47
316			412	578	---	528	18	62	---	112
317			494	722	524	433	36	68	66	89
		Av.	472	584	480	493	Av. 26	57	80	77
318	C	None	517	508	---	591	13	112	---	69
319			561	465	451	440	19	25	39	80
320			567	659	332	386	13	21	168	44
321			576	576	403	478	14	64	127	52
		Av.	555	552	395	474	Av. 15	56	111	61
322	A	100 mg.	312	829	1221	1489	8	31	229	62
323			364	855	1065	1475	16	35	165	155
324			441	723	965	2231	49	27	195	59
325			412	885	918	3826	88	45	232	124
		Av.	382	823	1042	2255	Av. 40	35	263	125
326	B	100 mg.	519	710	1198	2543	11	35	263	125
327			501	616	995	2073	29	44	175	57
328			317	545	1299	1632	33	25	151	178
329			435	665	1452	3743	65	35	218	97
		Av.	443	634	1211	2498	Av. 37	39	205	90
330	C	100 mg.	754	1192	1545	3252	36	48	295	278
331			494	693	1437	1407	26	27	193	103
332			804	590	1176	2770	26	60	334	112
333			636	728	1390	2858	24	52	180	94
		Av.	672	801	1387	2572	Av. 28	47	251	146

Table 5. Analysis of Muscle and Liver for Per Cent Fat

	Treatment	Average Total Lipid	
		Muscle	Liver
Phase I(a)	Restricted-injected	8.9	18.5
	Restricted-uninjected	6.9	13.7
	Unrestricted-injected	13.0	26.3
	Unrestricted-uninjected	12.8	22.2
Phase I(b)	Restricted-injected	9.5	18.0
	Unrestricted-injected	11.0	23.1

Results of Phase I(b) Showing Fatty Acid Levels
in
Starved and Full-fed Stilbestrol Injected 12 Week Cockerels



Blood Fatty Acid Mg.%

Fig. II

Results of Phase II Showing Fatty Acid Levels
in
12 Week Cockerels on Various Rations (A,B,C)

Blood Fatty Acids Mg.%

Fig. III

Results of Phase I(a) Showing Fatty Acid Levels
in
Starved and Full-fed Stilbestrol Injected 8 Week Cockerels

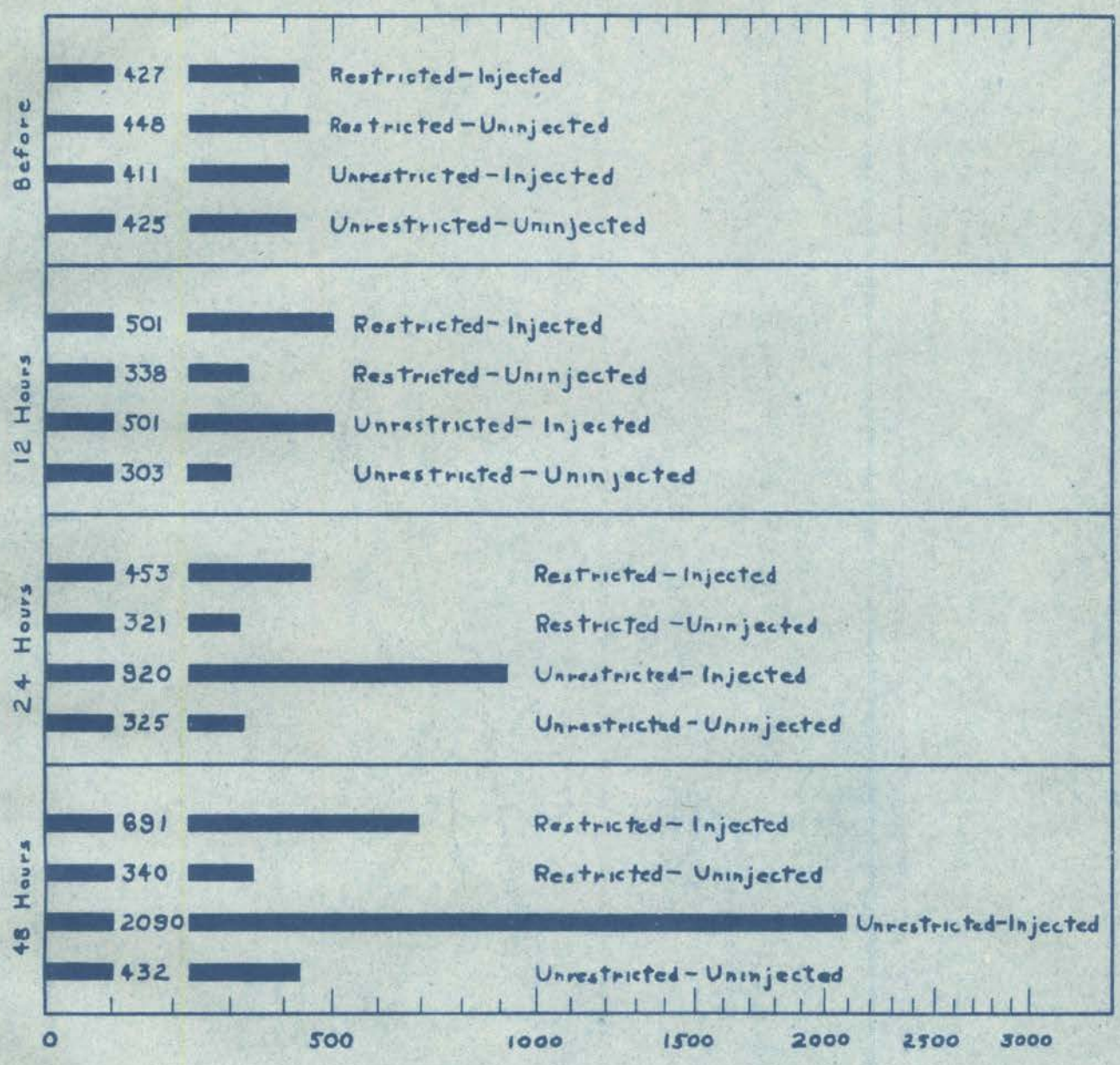


Fig. I

Discussion

Following the 48 hour bleeding of the birds in Phase I (a) and the 96 hour bleeding in Phase I (b), representative birds from each experimental group were sacrificed, muscle and liver samples were taken as described above and the entire carcasses inspected. Birds from Phase I (a) which had been on a restricted ration were uniformly emaciated, the fat of the viscera and beneath the skin having been virtually completely exhausted. Birds from Phase I (b) which had been on a less restricted diet were not nearly so wasted in appearance, although less fat was observed around the viscera.

Examination of the analysis of total tissue lipids (Table 5) showed that appreciable differences in both liver and muscle lipid were obtained in Phase I (a) between full-fed and restricted birds. Administration of estrogen did not produce any detectable change in tissue lipid during the experimental period. It should be emphasized, however, that the limited number of tissues analyzed precludes any final conclusions in this regard. In the group of birds of Phase I (b), which were all injected with diethyl stilbestrol, a marked decrease in liver fat was seen in the birds on a restricted ration while little differences were noted in liver fat. In view of these findings, it is felt that in both Phase I (a) and I (b) the technique used for depleting the body fat stores was satisfactory.

The technique of administering a single dosage of estrogen in oil has been used only rarely in experimental studies with chickens. For the purposes of these experiments, however, it

was thought that the response of the bird to a massive dose during a limited period of time would be more indicative of the nature of its physiological effect on lipid metabolism than would several smaller doses. Preliminary studies showed that the administration of a single 10 mg. dose to young growing cockerels did not produce the expected rise in total blood fat. The dosage was, therefore, increased to 100 mg. and further studies employed at this level.

Lorenz (16) has studied the effects of intramuscular implants of diethyl stilbestrol in chickens. During a four week period a total of 12.4 mg. of the drug were made available to the bird. This produced definitive changes in the secondary sexual characteristics of the birds and produced the usual increase in blood lipid. No explanation is offered as to the differences noted between these two methods of administration. It is possible that rapid absorption and excretion of the drug occurs when it is administered subcutaneously in oil, thus allowing its action over a relatively short period of time.

Following the administration of a single massive dose of diethyl stilbestrol, the blood lipid showed no change in 12 hours regardless of treatment (Phase I (a)); at 24 hours, however, a definite increase was found. The total blood fat of the control birds rose from an initial value of 411 to 920 mg. per cent. In the second experiment the increase was considerably greater, changing from 499 to 1275 mg. per cent. This is in accordance with the findings of Lorenz, Entenman and Chaikoff (17), who found after 24 hours a two-fold increase

in blood fat following the administration of 3000 rat units of natural estrogen in 1000 unit doses at 4 hour intervals. In these experiments, the blood fat increased still further during the period from 24 to 48 hours, increasing from 920 to 2090 mg. per cent in the first experiment and from 1275 to 2867 mg. per cent in the second. In the second experiment samples were collected at 72 and 96 hours; these definitely decreased, falling from 2867 mg. percent at 48 hours to 2005 and 1309 mg. per cent at 72 and 96 hours respectively. Several treated birds had blood fatty acid values within the normal range at 96 hours.

In general, whole blood cholesterol values showed small and inconsistent changes in full-fed and restricted birds in the absence of estrogen administration. Following such treatment, there was a tendency towards a slight cholesterolemia at from 24 to 72 hours. In experiment I (b), in which a larger number of birds were employed, it appeared that there was a trend toward higher values in the full-fed lot. Such changes were not statistically significant.

Further examination of the data in Tables 1 and 2, shows that under the conditions of this experiment no appreciable change in blood lipid was produced by inanition. It has been previously reported (18) that blood fat shows no change during starvation or may even increase.

In this experiment the birds were always receiving a substantial part of a maintenance ration and hence were not subject to such severe stresses. The average value for all the non-injected restricted birds in Phase I (a) was 362 mg. per cent with a range of from 321 to 448 mg. per cent.

Corresponding values for the full-fed lots were 371 mg. per cent with a range of 303 to 432 mg. per cent.

While inanition did not directly affect the blood lipid under these conditions, it did result in a dramatic change in the response to estrogen administration. Thus in Phase I (a) at 24 and 48 hours the full-fed birds had increased blood fat values of 920 and 2090 mg. per cent. Only a very slight change was noted in the partially starved group at 24 and 48 hours, (453 and 691 compared with initial and 12 hour values of 427 and 501 mg. per cent). In the second experiment where the degree of depletion was less severe, there was still a definite reduction in the degree of response of blood fat level to estrogen administration. A sufficient number of birds was included in this second experiment to permit a statistical treatment of the data. The data was analyzed by the method of variance (Snedecor, 19); differences in

Table 6

Analysis of Variance

Source	d.f.	S.S.	M.S.
Total	119	117,020,322	
Diet	1	18,456,655	18,456,655*
Time	4	39,379,905	9,844,976.2
dxt	4	6,371,632.9	1,592,908.2
Error	110	52,812,029.1	480,109.4

blood fat at different times did not reveal statistical significance ($F = 6.16$, $F_{.05} = 6.39$), although it will be seen that the value of F approached that required for significance

at the 5 per cent level. Difference due to treatment (restricted as compared with full-feeding) was, however, statistically significant ($F = 11.58$, $F_{.05} = 7.71$, $F_{.01} = 21.20$).

These data suggest that the principle source of the increased circulating blood lipid following hormonal stimulation by subcutaneous injection of estrogen is a mobilization of the fat from the depot stores. When these are partially or nearly completely eliminated, the effect is profoundly lessened.

Phase II was designed to approach the problem of the source of the increased circulating blood lipid in a different way than the previous experiments. Various levels of fat were supplied to the birds in the form of the three previously described partially purified rations. Ration A approximated a normal poultry ration with respect to fat content--4.8 per cent. Rations B and C were fortified with vegetable fat; the carbohydrate fraction was reduced correspondingly to maintain the three rations isocaloric. Despite the fact that ration C contained 26 per cent fat, none of the birds showed any reluctance to eat the ration at any time. They gained in weight normally. At no time during the period did any of birds "go off feed."

Examination of the data in Table 4 shows that there were no appreciable differences in blood fatty acid levels of the normal birds receiving varying levels of fat. Averages for all birds in each lot for all dates of sampling were as follows: Ration A, 476 mg. per cent (392 - 605); Ration B, 507 mg. per cent (400 - 722); Ration C, 494 mg. per cent (382 - 659). Changes in whole blood cholesterol were small and nor consistent.

These values suggest that at these levels of fat ingestion the chicken does not conform to the general pattern of an elevation of plasma or whole blood fatty acids with increasing fat content of the diet. It will require a larger number of animals to satisfactorally answer this question.

In contrast to the first two experiments, all of the birds injected with diethyl stilbestrol showed an increase in fatty acids at 12 hours. At 24 and 48 hours, still further increases were noted. At these later times there was a tendency for the birds receiving the highest level of fat to have slightly higher fatty acid values. The limited number of experimental birds and the wide variation in lipid content, however, precludes any conclusions being drawn. No cholesterolemia was produced under these conditions. It appears from these observations, that the level of fat in the ration *per se* has little to contribute to the degree of increase of fatty acids under estrogen treatment and further likely that the fat in the depots of the body make by far the largest contribution to the fat in the lipemic blood.

SUMMARY

The changes in the blood lipid levels of normal and estrogen treated cockerels on various rations have been investigated.

It has been demonstrated that no detectable variation is to be found in the blood fat levels of cockerels as the result of an inanition treatment. When such birds were treated with diethyl stilbestrol, only very slight increases were noted in the blood fat. When full-fed birds, however, were administered a single massive dose of this estrogen noticable rises in blood fat were found as early as 12 hours after injection with increases continuing until a peak value was reached at 48 hours, not infrequently 6 times the value in normal birds. It is therefore concluded that the depletion of body lipid stores by inanition greatly hampers the development of an estrogen induced lipemia.

Birds being maintained on a ration containing as much as 26 per cent fat, showed no tendency to develop a lipemic condition. When birds being fed rations containing 4.78, 13, and 26.3 per cent fat were injected with a single large dose of diethyl stilbestrol, dramatic increases in the blood fat were seen, with little differences apparent between birds on the three rations. Whereas the blood fat in birds on a normal chick ration rose to above 2800 mg. per cent on the average, following estrogen treatment, average values at 48 hours in the birds consuming high fat diets did not increase much above 2500 mg. per cent. Thus it is concluded that the level of fat in a chicken ration does not have a significant effect

on the increase in blood lipids due to estrogen administration. Furthermore it seems likely that the body fat stores are the chief source of the large amounts of blood fat in an estrogen induced lipemia.

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