allowed to grow as a spreading thin pellicle under static conditions. Biochemical and antibiotic sensitivity comparisons of AP− strains with their corresponding AP+ parent indicated no qualitative differences other than changes in pigmentation at 37°C. In addition, refrigeration of pigmented AP+ strains grown on tryptose agar lead to the appearance of pink pigmentation in many strains, whereas none of the stock AP− cultures ever exhibited this property.

1. Berk, R. S., J. Bact., 1963, v86, 728.
2. Berk, R. S., Gronkowski, L., A. van Leeuwenhoek, 1964, v30, 141.
3. Berk, R. S., Canad. J. Microbiol., 1965, v11, 213.
4. Döll, W., Freytag, K., Arch. für Mikrobiol., 1964, v48, 332.
5. Feary, T. W., Fisher, E., Fisher, T. N., J. Bact., 1964, v87, 196.
6. Hadley, P., J. Infect. Dis., 1924, v34, 260.
7. Haynes, W. C., Rhodes, L. J., J. Bact., 1962, v84, 1080.
8. Howarth, S., Dedman, M. D., ibid., 1964, v88, 273.
9. Jones, L. M., McDuff, C. R., Wilson, J. B., ibid., 1962, v83, 860.
10. Warner, P.T.J.C.P., Brit. J. Exp. Path., 1950, v31, 242.

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Some Effects of Ethanol on the Disposition of Palmitate by Intact And Adrenalectomized Rats. (31331)

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A single large dose of ethanol produces a triglyceride fatty liver in normal rats(1,2), but not in adrenalectomized (ADX) rats(3). Adrenalectomy also prevents the development of fatty livers after administration of other agents, such as carbon tetrachloride(4) and ethionine(5). Adipose tissue has been shown to be the main source of the fatty acids deposited in liver as triglycerides (TG) after ingestion of a single dose of ethanol in the intact rat(6). The free fatty acids (FFA) are liberated from adipose tissue and then are transported by the plasma to the liver where they are incorporated into more complex lipids. Since the ADX rat cannot mobilize FFA from adipose tissue as well as the intact rat(7,8), there may be an inadequate supply of FFA in ADX animals for synthesis of liver lipids. In addition, the liver of the ADX rat might extract less FFA from blood plasma or incorporate less FFA into liver lipids. We show in this paper that these last two possibilities are not probable explanations for the inability of ADX animals to develop TG fatty livers.

We have compared in intact and ADX rats the incorporation of labelled palmitic acid into liver lipids, its excretion as $C^{14}O_2$ and the effects produced by ethanol on these processes. The results obtained indicate that the effects of ethanol were qualitatively similar in the intact and the ADX rats.

Methods. ADX female Sprague-Dawley rats (Hormone Assay, Chicago, Ill.), weighing 160 to 180 g, were used. The ADX rats were given a 5% glucose-1% NaCl solution as drinking water until about 18 hours before use which was 4 to 7 days after surgery. All rats were fasted overnight before use, with access to drinking water (1% NaCl solution for ADX rats).

Ethanol, 4 or 6 g/kg as specified in *Results*, was given orally in a 50% (v/v) aqueous solution. Palmitate-1-C^{14} was injected 2 hours or 4 to 5 hours after administration of ethanol. Blood ethanol levels were determined using alcohol dehydrogenase and DPN (Boehringer blood ethanol assay).

Each rat was injected in the tail vein with palmitate-1-C^{14} (Nuclear-Chicago, 29.7 mC/mmole), 0.06 μC/g in a volume of about 0.25 ml. The palmitate was dissolved in a solu-

tion of 17% crystalline bovine plasma albumin (Armour)(9). Each rat received approximately 0.3 microequivalent (μeq) of the fatty acid.

Carbon dioxide was collected in methylbenzethonium chloride using an apparatus modified from that of Fredrickson and Ono (10). The methylbenzethonium chloride (Hyamine-10 X, Packard Instrument Co., LaGrange, Ill.) was counted in 0.4% diphenyloxazole in toluene and was titrated to determine the amount of CO_2 collected in milliequivalents (meq).

One hour after the injection of palmitate, each rat was stunned and the liver was removed and frozen. Hepatic lipid content was determined by the procedure of Chernick and Scow(11). Weighed liver slices (approximately 200 mg) were extracted with 10 ml of chloroform-methanol (2:1). An aliquot was placed in a 15 ml tube and evaporated to 1 ml at 60-70°C under N_2. About 0.5 g of activated silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pa.) was added, and the mixture was allowed to stand for 15 minutes. Following the addition of 2 ml of chloroform, the tube contents were poured into a 40 mm funnel plugged with glass wool. The tube was rinsed with several 2 ml portions of chloroform which were poured through the funnel to elute neutral lipids. The elution was complete when 10 ml had passed through the funnel. Phospholipids (PL) were eluted with 2 ml of chloroform-methanol (9:1) followed by small portions of methanol until 10 ml of eluate were obtained. After separation into neutral lipids and PL, aliquots of each were dried and then saponified with 2 ml of alcoholic KOH (1 ml of saturated KOH, freshly diluted to 20 ml with 95% ethanol). The samples were neutralized to the bromocresol green end point, diluted with water to 6 ml and extracted with 5 ml of n-hexane. One portion was titrated for fatty acid content with 0.002 N NaOH, and another was taken to dryness and counted. Specific activities were expressed as disintegrations per minute per microequivalent of fatty acid (dpm/μeq). Comparisons were made of total lipid fatty acid (TLFA), triglyceride fatty acid (TGFA), and phospholipid fatty acid (PLFA). Any FFA would be included in TGFA fraction. The contribution of FFA to TGFA in the liver was negligible since FFA levels in both ADX and intact rat livers were found to be less than 4.0 μeq/g.

Radioactivity was measured with a Packard Tricarb Liquid Scintillation Counter, using 0.4% diphenyloxazole in toluene as the scintillating solution. Disintegrations per minute (dpm) were calculated, using a C^{14} toluene (Packard Instrument Co.) internal standard to determine the counting efficiency.

Phospholipids were separated into their major classes by thin layer chromatography on silicic acid (Eastman chromagram sheets) emyloying chloroform:methanol:1.0 M sodium acetate, pH 3.55 (65:35:2, v:v:v). The spots were localized with iodine vapor, transferred into counting vials and counted in a polar counting solution (1500 ml toluene, 13 g 2,5-diphenyloxazole, 400 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene, 2500 ml ethylene glycol monomethyl ether)(12).

Results. The cumulative excretion of $C_{14}O_2$ within 1 hour after the intravenous injection of palmitate-1-C^{14} is illustrated in Fig. 1. The curve for intact control animals resembles that obtained by McCalla, Gates, and Gordon(14). There was no significant difference between untreated normal and ADX rats in their excretion of total or radioactive CO_2 (Table I). Ethanol reduced the excretion of labelled CO_2 by more than 50%. In the ADX rats, ethanol lowered the excretion of both the total and radioactive CO_2. This reduced total CO_2 excretion after ethanol reflects the depressed general metabolism of the ADX rat, associated with a marked anesthesia and blood levels of ethanol which were higher than those in intact rats (Table II). Masoro *et al*(13) found that the $C^{14}O_2$ yield from palmitate-1-C^{14} was not related to size of rat or to endogenous metabolism. The amount of FFA injected was increased 3-fold by using palmitate of lower specific activity (10 mC/mM). This amount did not change the excretion of labelled CO_2 significantly ($p>0.05$) in ADX rats (Table I).

The effects of ethanol on palmitate incorporation into liver lipids are shown in Table III. In untreated rats, the specific activities

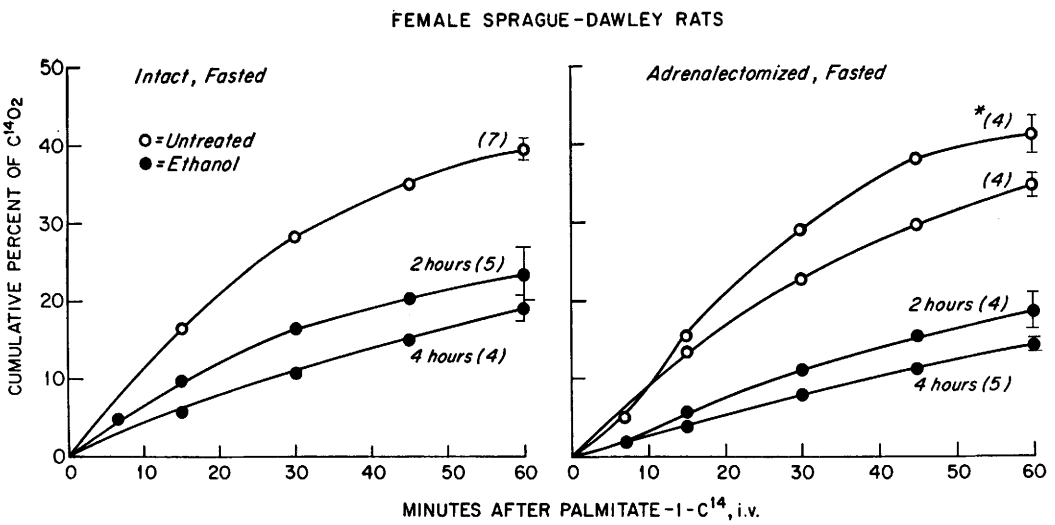

FIG. 1. The cumulative percent of injected dose of palmitate-1-C^{14} excreted in the expired air. The starred (*) group received 0.9 μeq FA as compared with 0.3 μeq FA for the other groups. The number of animals in each group is shown in parentheses.

TABLE I. Excretion of Carbon Dioxide During First Hour After Injection of Palmitate-1-C^{14}, Intravenously.*

	Intact rats		ADX rats	
Type of treatment	meq CO_2	% Dose C^{14}	meq CO_2	% Dose C^{14}
No treatment	9.56 ± .38 (7)	39.31 ± 1.56	8.71 ± .18 (4) (0.3 μeq FA)	34.43 ± 1.57
			9.30 ± .45 (4) (0.9 μeq FA)	40.85 ± 2.67
2 to 3 hr after ethanol	8.90 ± .50 (5)	†24.36 ± 1.62	6.80 ± .79 (4)	††17.77 ± 1.80
5 to 6 " " "	9.11 ± .34 (4)	†19.12 ± 1.45	††5.10 ± .27 (5)	††14.25 ± .78

* Values are means ± standard errors of mean. No. of rats in parentheses.
† Treated rats are significantly different from untreated rats ($p < .001$).
‡ ADX rats are significantly different from intact rats ($p < .05$).

of fatty acids in TG and PL were considerably higher in ADX than in intact rats (Table III, A). As early as 3 hours after administration of ethanol, the specific activity of TGFA increased approximately 3-fold in both intact and ADX rats. The specific activity of PLFA was increased about 50% in intact rats at 3 hours, but not at 6 hours. In the ADX rats, the specific activity of PLFA had increased about 30% by 3 hours and more than 2-fold by 5 hours.

TABLE II. Blood Levels of Ethanol in Fasted Animals After 4 g/kg, p.o.

	Ethanol content of blood (mg/100 ml)		
Hours after ethanol	1	2	4
Intact	220	292	295
ADX*	436	397	357

* These animals were kept on 5% glucose-1% saline drinking water.

Total liver lipids were lower in untreated ADX rats than in intact rats (Table III, B). Livers from untreated ADX rats contained only one-half as much TGFA and three-quarters as much PLFA as livers from intact rats. Over a period of 5 to 6 hours, ethanol increased the TGFA almost 3-fold in the intact animals, without producing a significant increase in the ADX rats. At 3 hours, ethanol increased the amount of liver PLFA in both intact and ADX rats. By 5 hours, however, the amount of PLFA had returned to untreated levels in ADX rats and had fallen

TABLE III. Effect of Ethanol (4 g/kg Orally) in Intact and ADX Rats on Incorporation of Palmitate-1-C^{14} into Liver Lipids.*

	Untreated		Hours after ethanol: 3 hr		5 to 6 hr	
	Intact (4)	ADX (6)	Intact (6)	ADX (7)	Intact† (4)	ADX (5)
A. Specific activity of liver lipids (dpm/μeq)						
TLFA	3,490 ± 400	‡6,070 ± 650	§6,960 ± 585	‡§10,150 ± 808	§8,270 ± 394	‡§15,350 ± 1,880
TGFA	3,230 ± 425	‡6,260 ± 610	§9,220 ±1,606	‡§17,420 ±2,460	§9,760 ± 470	‡§17,760 ± 2,520
PLFA	3,230 ± 235	‡5,740 ± 590	§4,826 ± 315	§‖ 7,500 ± 580	3,780 ± 145	‡§12,040 ± 1,660
B. Total liver lipids (μeq/liver)						
TLFA	556 ±126	419 ± 15	636 ± 40	‡481 ± 18	§978 ± 80	‡425 ± 36
TGFA	265 ± 40	‡132 ± 34	264 ± 36	‡104 ± 17	§767 ± 62	‡211 ± 32
PLFA	344 ± 18	‡254 ± 26	§470 ± 25	§472 ± 18	§188 ± 14	206 ± 37
C. Percent of palmitate-1-C^{14} incorporated into the entire liver						
TLFA	8.18 ±1.44	‡13.07 ± 1.10	§22.20 ± 1.86	§22.58 ± 1.64	§25.24 ± 2.10	§30.20 ± 2.21
TGFA	3.82 ± .62	4.40 ± 1.44	§11.75 ± 1.67	‡ 7.61 ± .72	§23.25 ± 1.97	‡§17.29 ± 2.71
PLFA	5.22 ± .82	8.47 ± 1.01	§11.45 ± 1.00	‡§16.33 ± 1.00	§ 2.24 ± .26	‡11.62 ± 2.28

* Mean values ± S.E.M.
† These 4 rats received 6 g/kg ethanol.
‡ ADX rats significantly different from intact rats ($p < .05$).
§ Treated rats significantly different from untreated rats ($p < .05$).
‖ Treated rats *vs* untreated, $.05 < p < .10$.

TABLE IV. Distribution of C^{14} in Liver Phospholipid Classes.*

	Untreated		Ethanol treated				
Class	Intact	ADX	Intact 2 hr 4 g/kg	ADX 2 hr 4 g/kg	ADX 4 hr 4 g/kg	Intact 4 hr 6 g/kg	ADX 4 hr 6 g/kg
Lysolecithin	1.7	6.0	—	—	4.6	14.3	16.0
Sphingomyelin	3.9	5.2	3.2	4.6	4.2	6.4	8.0
Phosphatidylcholine	64.3	59.6	60.4	61.4	55.8	27.4	26.5
Phosphatidylserine	5.2	5.2	5.7	6.9	7.7	24.7	27.8
Phosphatidylethanolamine	22.3	21.0	22.1	22.5	25.7	13.8	14.5
Cardiolipin	2.0	2.2	6.7	2.7	1.1	5.6	4.7

* Values are in percent of total label in the mixture. All animals were injected with palmitate-1-C^{14} in albumin at the indicated time after ethanol. They were killed 1 hr later.

about 65% below untreated levels in intact rats.

The distribution of palmitate-1-C^{14} in the major phospholipid classes is summarized in Table IV. Results are expressed as per cent of the total counts in PL recovered in each fraction. Adrenalectomy alone did not change the distribution of radioactivity among the various fractions. At 3 hours, ethanol did not alter appreciably the distribution of label among the PL classes in either intact or ADX rats. Five hours after a dose of 6 g/kg of ethanol, however, in both intact and ADX rats the palmitate C^{14} content of phosphatidyl serine had increased strikingly, with a concomitant decrease in phosphatidyl choline label. This redistribution of C^{14} among the PL classes indicates that a large dose of ethanol can affect the metabolism of some phospholipids markedly.

Discussion. The amount of ethanol given to our animals is less than doses used in many other laboratories which range up to 7.5 g/kg, p.o. We lowered the dose from 6 g/kg to 4 g/kg when we found that some ADX animals died within 5 hours after the larger amount. Normal animals did not die after a 6 g/kg dose. The greater toxic effect of ethanol in ADX animals is commensurate with the higher blood ethanol levels in ADX rats (Table II).

We think that the main source of $C^{14}O_2$ from our injected FFA is the liver, even though muscle and adipose tissue can oxidize FFA to CO_2. Geyer *et al*(15) found about 8% of palmitic acid going to $C^{14}O_2$ in

the first hour after administration to an eviscerated rat. This is a maximal figure for peripheral metabolism because removal of the liver elevates plasma levels of FFA promptly (16). After 5 minutes, very little palmitate-1-C^{14} is present in an unesterified form, so for most of the one-hour period we are measuring the metabolism of glycerides. Many studies indicate that glyceride metabolism, under our conditions, should take place largely in the liver. Both fasting and adrenalectomy accelerate the removal of TG from the plasma by rat liver, as shown by studies on the perfused liver(17). Studies in the dog(18) have shown that the hepatectomized animal derives little $C^{14}O_2$ from tripalmitin-C^{14}, i.v. Other workers have shown that plasma PL are formed in the liver(19) and return to the liver for breakdown(20).

The 50% decrease in conversion of palmitate-1-C^{14} to $C^{14}O_2$ (Fig. 1, Table I) indicates that ethanol decreases oxidation of FFA to CO_2 *in vivo* both in intact and ADX rats. Rebouças and Isselbacher(21) reported a similar depression in the labelling of respiratory CO_2 from palmitate-1-C^{14} given intraperitoneally to fed intact rats 4 hours after ethanol. A sparing effect of ethanol on the oxidation of palmitate by liver slices from normal rats has also been reported by Forsander *et al*(22). Since this depression of FFA oxidation by ethanol was as great in ADX rats as in intact rats, it probably is not a major factor in producing the ethanol-induced fatty liver in intact rats. Depressed FFA oxidation could contribute appreciably, however, to the higher per cent label found after ethanol in liver TGFA and PLFA in both intact and ADX rats (Table III, C).

The plasma FFA levels of fasted ADX and intact rats are about 0.7 μeq/ml(23). Increasing the amount of injected labelled FFA from 0.3 μeq to 0.9 μeq did not change the per cent palmitate oxidized to $C^{14}O_2$ by ADX rats (Table I). This finding justifies our assumption that the amount of fatty acid injected in our rats was a tracer amount.

Administration of ethanol to fasted normal rats has little effect on plasma FFA levels as shown by Elko *et al*(24) who report levels of 0.73 and 0.74 μeq/ml, 2 and 4 hours after saline treatment as compared with 0.71 and 0.70 μeq/ml 2 and 4 hours after 6 g/kg of ethanol orally. Maickel and Brodie have shown that ethanol does not change the plasma FFA level in ADX rats(25).

Despite the decreased content of liver TGFA and PLFA in the ADX rat (Table III, B), the per cent label found in liver TLFA was higher in the ADX rat than in the intact rat. Obviously, there is no defect in the ability of the ADX rat to incorporate FFA into more complex lipids.

We have demonstrated an increase in labelled fatty acid content of liver glycerides as early as 2 to 3 hours after ethanol in both normal and ADX rats. At this time, no net increase in liver triglycerides is apparent.

Our measurements of liver lipids were made 1 hour after injection of labelled palmitate-1-C^{14} because we collected CO_2 from the same rats. Although some redistribution of label has occurred, the variability from rat to rat is much less at 1 hour than during the first 10 minutes(26). In both normal and ADX rats, ethanol decreased the excretion of labelled CO_2 to about the same extent as it increased the one-hour content of label in liver lipids (compare per cent dose values in Tables I and III). Our data do not conclusively indicate that maximal uptake by the liver is increased by ethanol. The data do eliminate the possibility that a decreased incorporation of FFA into liver lipids may be a contributing factor in the failure of ethanol to induce a fatty liver in ADX rats.

Ethanol increased liver TGFA in intact rats. Ethanol in the intact rat causes release of corticoids(2) which stimulate the liver to greater TG synthesis. Hays and Hill(27) found that corticoids stimulate the formation, *in vitro,* of enzymes for TG synthesis. This finding could explain some of the TG accumulation in intact animals. In the ADX animal, we found increased label in the TGFA without increased amounts of TGFA. This relative increase in FFA-C^{14} utilization for liver lipid synthesis could be due to a decrease in dilution by endogenous FFA or might reflect a shift in the lipid compartments of the liver.

Ethanol increased the PLFA content of

the liver at 3 hours, but decreased the content at 5 hours. Scheig and Isselbacher(28) report experiments in rat liver slices from ethanol-treated rats which show a 100% increase in palmitate conversion to TG with a reciprocal decrease in PL formation. Maling, Wakabayashi and Horning(29) have also reported an increased incorporation by liver homogenates from ethanol-treated rats of labelled palmitate into TG and a decreased incorporation into PL.

In both intact and ADX rats, ethanol increased incorporation of label into liver PLFA 3 hours after ethanol. Five hours after ethanol, the per cent label in liver PLFA was reduced in intact rats. In ADX rats the liver PLFA at this time contained slightly more label than in untreated rats. It would appear that after ethanol, the demand for TG synthesis eventually overwhelms and exhausts the metabolic supplies for other routes such as PL synthesis. On the other hand, these data could indicate that a marked increase in PL utilization had been induced, perhaps for FFA oxidation or for TG synthesis.

The changes observed in the phosphatidylserine and phosphatidylcholine fractions are further indications of the diverse changes in PL metabolism which occur after large doses of ethanol. Since PL are important components of the cell membranes and of lipoproteins, significant changes in PL metabolism could readily affect the deposition of TG in the liver and the transport of TG out of the liver as lipoproteins.

Summary and conclusions. Our experiments show that adrenalectomized rats oxidize FFA and incorporate FFA into liver lipids as well as the intact rat. In both groups of animals, ethanol increased the content of palmitate-1-C^{14} in liver lipids at 1 hour and decreased the amount oxidized to CO_2. The most marked increase in isotope content of liver lipids occurred in the ADX rats. Among the effects of large doses of ethanol on PL classes was a marked relative increase of palmitate incorporation into the phosphatidylserine fraction of both groups and a corresponding decrease in the amount in phosphatidylcholine. Blood levels of ethanol were higher in adrenalectomized rats than in intact rats.

1. DiLuzio, N. E., Am. J. Physiol., 1958, v194, 453.
2. Brodie, B. B., Butler, W. M., Jr., Horning, M. G., Maickel, R. P., Maling, H. M., Am. J. Clin. Nutr., 1961, v9, 432.
3. Mallov, S., Bloch, J. L., Am. J. Physiol., 1956, v184, 29.
4. Calvert, D. N., Brody, T. M., ibid., 1960, v198, 669.
5. Wool, I. G., Goldstein, M. L., ibid., 1953, v175, 303.
6. Horning, M. G., Williams, E. A., Maling, H. M., Brodie, B. B., Biochem. Biophys. Research Communications, 1960, v3, 635.
7. Shafrir, E., Sussman, K. E., Steinberg, D., J. Lipid Res., 1960, v1, 459.
8. Maickel, R. P., Brodie, B. B., Ann. N. Y. Acad. Sci., 1963, v104, 1059.
9. Fredrickson, C. S., Gordon, R. S., Jr., J. Clin. Invest., 1958, v37, 1504.
10. Fredrickson, C. S., Ono, K., J. Lab. Clin. Med., 1958, v51, 147.
11. Chernick, S. S., Scow, R. O., J. Biol. Chem., 1964, v239, 2416.
12. Beaven, M. A., Maickel, R. P., Biochem. Biophys. Research Communications, 1964, v14, 509.
13. Masoro, E. J., Valentine, M. D., Felts, J. M., Proc. Soc. Exp. Biol. and Med., 1958, v98, 290.
14. McCalla, C., Gates, H., Gordon, R., Arch. Biochem., 1957, v71, 346.
15. Geyer, R. P., Waddell, W. R., Pendergast, J., Yee, G. S., J. Biol. Chem., 1951, v190, 437.
16. Borgstrom, B., Olivecrona, T., J. Lipid Research, 1961, v2, 263.
17. Heimberg, M., Weinstein, I., Klausner, H., Watkins, M. L., Am. J. Physiol., 1962, v202, 353.
18. Goldman, D. S., Chaikoff, I. L., Reinhardt, W. O., Entenman, C., Dauben, W. G., J. Biol. Chem., 1950, v184, 719.
19. ———, ibid., 1950, v184, 727.
20. Entenman, C., Chaikoff, I. L., Zilversmit, D. B., ibid., 1946, v166, 15.
21. Rebouças, G., Isselbacher, K. J., J. Clin. Invest., 1961, v40, 1355.
22. Forsander, O. A., Rahiha, N., Salaspuro, M., Maenpaa, P., Biochem. J., 1965, v94, 259.
23. Stern, D. N., Maickel, R. P., Life Sciences, 1963, v11, 872.
24. Elko, E. E., Wooles, W. R., DiLuzio, N. R., Am. J. Physiol., 1961, v201, 923.
25. Maickel, R. P., Brodie, B. B., Ann. N. Y. Acad. Sci., 1963, v104, 1059.
26. Brown, E. A., Hunter, J. M., Maling, H. M., The Pharmacologist, 1964, v6, 173.
27. Hays, A. P., Hill, R. B., Jr., Biochim. Biophys. Acta, 1965, v98, 646.

28. Scheig, R. L., Isselbacher, K. J., J. Clin. Invest., 1963, v42, 975.

29. Maling, H. M., Wakabayashi, M., Horning, M. G., Advances in Enzyme Regulation, 1963, v1, 247.

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Independent Influence of Chronic Hypoxia and Sexual Development Upon Circulating Erythrocyte Concentration in Male Chickens.* (31332)

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Two conditions are rather systematically associated with increases in circulating red cell concentrations in homeotherms—"maleness" and hypoxia. At sexual maturity, the hematocrit of male mammals becomes greater than that of females(1), and similar changes also occur in the domestic fowl(2). It also has been demonstrated that exogenous androgens have an erythropoietic stimulus in mature mammalian females and male castrates, but not necessarily in sexually mature males (3). The polycythemia of animals living at high altitudes was reported by Viault in 1890, and this has been the subject of abundant study since that time(4). Grant and Root (5) have concluded that hypoxia is the fundamental stimulus for erythropoiesis. However, this response to hypoxia is apparently restricted to homeotherms, and has not been found in poikilothermic animals(6).

The additive (and apparently independent) nature of these two polycythemia-inducing conditions was demonstrated in experiments at high altitude† and at sea level with cockerels, some of which were subjected to repression of sexual development by stilbestrol (estrogen) treatment.

Materials and methods. The experiment was conducted on White Leghorn cockerels of sea level stocks—*i.e.*, not the strain selected for high altitude(7). These were brooded and subsequently pen-reared under ordinary and similar husbandry conditions, and were fed a nutritionally adequate ration. At an age of 2 days, some of the cockerels were taken to an elevation of 10,150 feet, and the remainder retained at sea level. At 45 days age, the high altitude group was taken to an elevation of 12,500 feet, where they remained until termination of the experiment.

At 5 and 45 days of age, some birds of each group (high altitude and sea level) were implanted subcutaneously with one or two 12 mg stilbestrol pellets. This resulted in 5 treatment sub-groups at each elevation, which were designated according to number of pellets and time of their administration:

0-0: no treatment (*i.e.*, treatment controls);
0-1, 0-2: at 45 days of age birds were implanted with either one or two stilbestrol pellets; and,
1-1, 1-2: all these birds received a pellet at 5 days of age and either one or two stilbestrol pellets at 45 days of age.

Records were kept of mortality, and periodically body weights were determined. At approximately 24 weeks of age, the birds were sacrificed and measurements were made of testes weight and hematocrit (by the microcapillary method). Mean data were calculated for each group, and the significance of differences between them was estimated by Student's t-test(8).

Results. 1. *Growth and mortality.* The body weights of the various groups of experimental birds are summarized in Table I.

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† These studies were carried out at the White Mountain Research Station (near Bishop, Calif.), which has laboratories at 4,000, 10,150, 12,500 and 14,250 feet elevations. For further information regarding these facilities contact the Director: Prof. Nello Pace, Dept. of Physiology, Univ. of California, Berkeley.