TABLE III.
Extraction of Cholesterol of Lyophilized Normal Human Serum.

Subject	Cholesterol extracted in 3 hr, mg%	24 hr, mg %	27 hr, mg %	96 hr, mg %	Total Chol., mg %	Neutral fat + Chol., mg %
A.L.F.	20	20	25	—	227	428
D.F.M.	30	31	35	—	322	511
C.J.R.	28	25	27	—	270	428
A.F.	22	22	20	—	226	404
D.F.	22	29	28	—	240	594
A.B.	30	22	28	—	286	392
K.N.	20	26	21	23	215	533
G.W.J.	22	27	27	38	272	551
R.B.	34	38	37	35	272	735
G.C.	37	37	42	43	331	705
E.R.	27	42	55	—	267	627
L.S.	36	76	102	—	233	722
M.D.	24	24	32	—	210	365

chickens is being investigated.

Although the data which have been presented are not sufficient to enable one to draw conclusions as to the physical state in which cholesterol exists in serum, they do indicate that it probably exists in at least three states not related to the usual classification of free and ester cholesterol. These are (a) a fraction not extracted by chloroform under the conditions used; (b) a fraction which is readily extractable by chloroform, a 3 hour extraction period being adequate for its complete extraction; and (c) a fraction which is slowly extractable. We believe that (a) possibly represents cholesterol which is held firmly by the serum proteins, while (b) possibly represents cholesterol which is free in the serum or which is associated with other lipid materials such as fats and phospholipids. Our experimental evidence indicates that all the fractions contain both free and ester cholesterol.

Summary. A large percentage of the cholesterol of lyophilized serum from old hens is present in the "readily extractable" fraction. The concentration of this fraction in the serum of roosters and young chickens is quite low but, in some cases, the amount extracted increases with increased extraction time. There appears to be a correlation between the percentage of the total cholesterol present in the "readily extractable" fraction and the neutral fat content of the serum of old hens.

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17068. Acetaldehyde Disappearance and Acetoin Formation *in vitro*.*

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The presence of a diphosphothiamin enzyme in animal tissues capable of forming acetoin from pyruvate, and the utilization of acetaldehyde as a substrate in this reaction, has been described.[1] A limited study of this reaction, in brain homogenate was reported previously.[2] The present study was devoted to a comparison of the abilities of various tissues to meta-

* Aided by a grant from the Nutrition Foundation, Inc.

1 Green, D. E., Westerfeld, W. W., Vennesland, B., and Knox, W. E., *J. Biol. Chem.*, 1942, **145**, 69.

2 Stotz, E., Westerfeld, W. W., and Berg, R. L., *J. Biol. Chem.*, 1944, **152**, 41.

TABLE I.
Acetaldehyde Disappearance and Acetoin Formation *in Vitro* with Rat Tissues.

Substrate	Micromols added	Brain		Leg muscle		Liver	
		AcH disapp., γ	Acetoin formed, γ	AcH disapp., γ	Acetoin formed, γ	AcH disapp., γ	Acetoin formed, γ
None	—	71*	36*	71	69	144	21
Pyruvate	3	95*	95*	124	164	142	37
Glucose	6	92†	100†	68	76	144	23
α-Ketoglutarate	6	89	67	125	124	140	0
Lactate	12	73	58	64	73	142	0
Alanine	12	75	58	69	71	141	0
Phosphoglycerate	6	70	50	96	107	143	0
Citrate	6-100	71	28	47	37	147	0

* Approximately the same results were obtained in oxygen and nitrogen.

† Approximately the same results were obtained in oxygen; in nitrogen the acetaldehyde disappearance was similar but the acetoin formation was only 46 γ.

The reproducibility of the results is indicated by the following data for 11 experiments with rat brain in the absence of substrate. Acetaldehyde disappearance: mean = 71, S.D. = 10.3, S.E. = 3.1, range = 57-88. Acetoin formed: mean = 36, S.D. = 3.55, S.E. = 1.07, range = 31-42. The effect of each substrate was compared with a simultaneous control in 2 or more experiments.

bolize acetaldehyde and form acetoin, and to the effect of added substrates upon these reactions.

Methods. To a Warburg vessel were added 1 cc of tissue homogenate in saline containing 250 mg of fresh tissue, 1.5 cc of 0.1 M phosphate buffer, pH 7.4, 20 γ diphosphothiamine in 0.1 cc water, 0.2 cc of water or substrate solution, and 0.3 cc of 0.05% acetaldehyde in the side arm. After 10 minutes equilibration at 37.5°C, the acetaldehyde was tipped in and shaken for 1 hour. A tungstic acid filtrate of the contents of the cooled flask was then prepared and analyzed for the acetaldehyde remaining[3] and the acetoin formed.[4] Mechanical and volatilization losses were small in the absence of tissue.

Results. Some of the results with rat tissues are shown in Table I. Brain and muscle breis removed acetaldehyde at about the same rate, but muscle formed twice as much acetoin as brain. Kidney brei in the absence of added substrate was similar to brain. Liver metabolized acetaldehyde at a much faster rate, but formed little acetoin. Corresponding results were previously obtained *in vivo*[5] where large amounts of acetoin were formed from acetaldehyde only when the dominant role of the liver in acetaldehyde metabolism was eliminated by hepatectomy.

The addition of pyruvate, or other substrates capable of forming pyruvate in the brei, increased both the acetaldehyde disappearance and the acetoin formation. Pyruvate and α-ketoglutarate gave large increases with both brain and muscle; glucose was also very effective in brain, but not muscle, while phosphoglycerate stimulated the reaction somewhat more in muscle than in brain. In liver, acetoin formation disappeared completely in the presence of most substrates.

Other results obtained with 6-12 micromols of substrate added to brain were: significant increases (30-60%) in acetoin formation from glycogen, lactate, alanine, phosphoglycerate and malate; minor increases (10-25%) from succinate, fumarate, glucose-l-phosphate, fructose and glycerol phosphate; no effect from butyrate, acetate, propionate, citrate, glutamate, cis-aconitate, galactose and glycerol. Anesthetization of the rat with nembutal or the addition of nembutal *in vitro* did not affect the reaction in brain tissue. The addition of 0.5 mg methylene blue to brain tissue increased the acetaldehyde disappearance 50% and the acetoin formation by 150%. When added together with pyruvate or glucose, methylene blue increased both the acetaldehyde disappearance and acetoin formation be-

[3] Stotz, E., *J. Biol. Chem.*, 1943, **148**, 585.

[4] Westerfeld, W. W., *J. Biol. Chem.*, 1945, **161**, 495.

[5] Lubin, M., and Westerfeld, W. W., *J. Biol. Chem.*, 1945, **161**, 503.

yond that obtained with either methylene blue or the substrate alone.

It is reasonable to suppose that the increased acetoin formation in the presence of added substrate represented an increased amount of pyruvate available for condensation with the excess acetaldehyde. The ineffectiveness of other members of the citric acid cycle, as compared with α-ketoglutarate, the effect of glucose in brain as compared with muscle, and the comparison of glucose with fructose or glucose-l-phosphate are noteworthy considerations in any interpretation of these results.

Further studies on the disappearance of acetaldehyde in liver brei were made with 0.3 cc of 0.15% acetaldehyde in the side arm. Rat liver removed about 200 γ acetaldehyde during the incubation; added glucose had no effect. Small increases (10%) in the amount removed resulted from the addition of pyruvate, lactate or alanine. 100 μM of citrate or 0.5 mg methylene blue increased the acetaldehyde disappearance by 25% and 40%. Dog liver gave similar results except that methylene blue had no effect. The methylene blue effect in rat liver as contrasted with dog liver is possibly related to the presence of xanthine oxidase in rat liver only; methylene blue stimulates aerobic oxidation by xanthine oxidase.

Summary. In agreement with previous *in vivo* studies, liver homogenate metabolized acetaldehyde more rapidly than other tissues and without the formation of much acetoin. Methylene blue stimulated the aerobic metabolism of acetaldehyde in rat liver but not dog liver.

Skeletal muscle formed more acetoin from acetaldehyde than did brain or kidney. Pyruvate, α-ketoglutarate and phosphoglycerate increased the acetoin formation from acetaldehyde by muscle; pyruvate, α-ketoglutarate and glucose were effective stimulators of acetoin formation in brain.

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17069. Isolation of an L Type Culture from a Gram Positive Spore-Bearing Bacillus.*

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During the last 10 years L type cultures have been isolated from many Gram negative bacilli. The author tried repeatedly to apply to Gram positive bacilli the methods which proved successful in Gram negative species. Success was obtained first in the summer of 1948 with a Gram positive bacillus isolated from a contaminated blood culture. The broth cultures of this bacillus were pleomorphic. After 24 - 48 hours of incubation almost all bacilli swelled to round bodies as in the cultures of some Bacteroides strains. This pleomorphic culture transferred to media of various compositions produced only bacterial growth, but when a penicillin cup was placed on soft horse-serum agar plates and the plates were incubated anaerobically, a few microscopical L type colonies developed near the edge of the zone of inhibition. Growth of these tiny colonies in pure culture was obtained by cutting out a block of agar containing the colonies and transferring it to horse serum agar containing 400 units of penicillin per cc. The colonies grew without difficulty in subsequent subcultures.

Following this observation several samples of dust and earth were planted on similar plates containing varying concentrations of

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