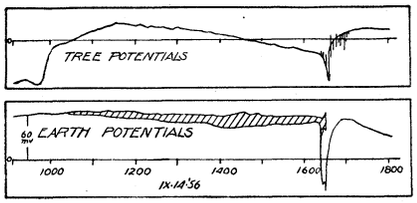

Fig. 1. Tree and earth potentials (millivolts) during a severe storm (Eastern Standard Time). The potential difference above the base line is positive for the upper electrode in the tree and for the south electrode in the earth.

A test run on earth potentials was started last summer, using exactly the same technique as was used in the tree, with silver-silver chloride electrodes buried in the moist earth, high-impedance input amplifiers, and photoelectric recorders. It is thus possible to obtain continuous and simultaneous records of the changing potentials.

On Friday, 14 Sept. 1956, between 4 and 6 P.M., Lyme, Conn., as well as many other New England areas, was hit by a severe squall. Very high winds and torrential rain, lasting for roughly half an hour, were observed. Examination of the photoelectric records from both the tree and the earth showed the rather remarkable phenomenon shown in Fig. 1. In the tree, the upper of the two electrodes was positive 40 or 50 mv, and in the ground the south electrode on the north-south axis was positive about 60 mv for several hours before the onset of the storm.

Prior to the onset of the storm, there was, for 4 or 5 hours in the earth record, an oscillation of the standing potential, the 10-mv envelope of which appears in Fig. 1 (cross-hatching). Then, quite suddenly, the positive potential of the south electrode dropped to zero and became the negative potential of 20 or 30 mv. As the storm passed, this excursion was reversed, and the relatively steady-state standing potential reappeared as a positive potential of 60 mv. During the succeeding 3 or 4 hours, the magnitude of this potential decreased to that characteristic of the early hours of the day.

A very similar change occurred in the tree potentials prior to the storm, with the development of a reversed polarity paralleling that in the earth, beginning somewhat sooner, and taking a little longer to develop.

Since this is a pilot experiment, no interpretation can be made at this time, but it is hoped to continue these studies, including not only north-south potentials in the earth, but also east-west potentials. As soon as funds are available with which to install the necessary equipment, atmospheric potentials will also be recorded simultaneously. Since it has been adequately demonstrated that a living organism, the tree, is an electric system exhibiting all the properties of an electric field, one may reasonably expect that changes in the electric environment will show some interrelationship with the field properties of the tree. Over many years, for example, thunderstorms have shown characteristic changes in the standing potential of the tree. Sharp spikes appear in the tree record during the storm. It should be noted that corresponding spikes did not occur in the earth record. Adequate controls have shown that these are not instrumental artifacts caused by interference in the power supply. Instead, they are evidence of very considerable changes in the tree potential associated with the very profound changes in earth and atmospheric electricity.

H. S. BURR

Section of Neuro-Anatomy,
Yale University School of Medicine,
New Haven, Connecticut

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Isolation of a Cardiac-Active Principle from Mammalian Tissues

It is known that the isolated mammalian heart, even though it is maintained under physiological conditions, gradually loses contractile force. This failure, which in many respects resembles chronic congestive heart failure, can be reversed by digitalis. It can also be overcome by inclusion of the liver in the circulatory pathway of the mammalian heart lung preparation (*1*). Failure of the isolated frog heart that has been induced by perfusion with Ringer's solution can be reversed by addition of mammalian serum to the heart (*2*, *3*). These observations have suggested the possible presence in mammalian tissues of material that resembles digitalis in its physiological effects (*1*, *3*).

A search was undertaken for the active principles in mammalian tissue, using a bioassay based on the staircase phenomenon in the frog heart (*4*). Deproteinized acetone extracts of tissue were defatted with petroleum ether, and the active material was fractionated by chromatography on Florisil. The most abundant source examined was beef adrenal medulla, which contained activity equivalent to 1000 μg of strophanthidin per kilogram; others were beef liver, 200 μg/kg and plasma, 180 μg/kg. Little or no activity was found in adrenal cortex, red blood cells, or skeletal muscle.

The active component has been crystallized from extracts of adrenal tissue and identified as monopalmitoyl glycerylphosphorylcholine (palmitoyl lysolecithin). On a molar basis, this material had 1/60 the activity of strophanthidin, 1/40 the activity of digitoxin and one-third that of digitoxigenin. Several lysolecithins prepared synthetically (*5*) have also been shown to have activity qualitatively similar to that of digitalis on the frog heart.

In addition to the known hemolytic activity of lysolecithin, the following biological effects of the isolated material have been observed. It increases the tension of the hypodynamic frog heart, abolishes the staircase phenomenon in the frog heart, increases the tension of the isolated squab ventricular muscle, and causes contracture of the isolated carotid artery strip. In all these respects, the action of digitalis is similar. Furthermore, this lysolecithin resembles the glycosides by virtue of its strong affinity for the heart, a quality not shared by other substances, such as the catecholamines and certain cortical steroids that have also been shown to be cardiotonic in the frog heart (*3*).

Although the isolated material appeared to be chemically pure, it exerted two biological effects; (i) the characteristic digitalis-like action on the staircase and (ii) a toxic activity resembling that of the saponins and characterized by rapid onset of contracture. The former effect was found to be associated with β-lysolecithin, while the toxic factor was identified as an isomer arising by intramolecular rearrangement of the ester bond to the α-carbon of the glycerol. The toxic substance appears to be an artifact formed during isolation. It may be destroyed by hydrolysis with the phospholipase A of *Crotalus adamenteus* venom.

Approximately 50 percent of the active substance in the adrenal medulla and 80 percent or more of that in liver and serum occurs in the form of an inactive precursor, which is somewhat more lipoid soluble than the lysolecithin and from which the latter is liberated on standing for a few hours at *p*H 2. This precursor appears to be a hemiacetal derivative of the lysolecithin with a long-chain fatty aldehyde. A substance of this structure has been identified in heart muscle by Klenk and Debuch (*6*).

What role palmitoyl lysolecithin might play in normal mammalian physiology cannot be decided on the basis of tests in the frog heart. If the mechanism by which it acts on the staircase phenomenon in this heart is the same as that of the cardiac glycosides, it would appear to serve as a regulator of membrane permeability to potassium (*4*).

The hemiacetal precursor might serve as a nonhemolytic form in which lyso-

lecithin could be transported to a site of action for release by liberation of the aldehyde. Whether such a site of action would be the heart or some other organ or organs cannot yet be decided. These questions are being investigated further.

ELWOOD TITUS
HERBERT WEISS
STEPHEN HAJDU

National Heart Institute,
National Institutes of Health,
Bethesda, Maryland

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27 September 1956

Evidence for Nonexistence of D-Aspartic Acid in Casein

The values reported previously for L-aspartic acid and total (L + D) aspartic acid in acid hydrolyzed casein (*1*) imply that the aspartic acid in this material was approximately 7 percent racemic (*2*). The question of whether racemization to this extent is a consequence of the hydrolysis procedure or whether D-aspartic acid residues are initially present in intact casein was left unsettled but appears now to be resolved by the results of the present experiments (*3*).

Samples (3 g each) of casein (*4*) were heated with 30-ml portions of 6*N* hydrochloric acid under reflux for varying periods of time, and each hydrolyzate was assayed for L-aspartic acid and for total aspartic acid by the previously described methods (*1*). The L-aspartic acid values (*5*) found after heating for 29, 32, 340, 550, and 720 hours, respectively, were 6.65, 6.92, 4.52, 4.00, and 3.77 percent, whereas the corresponding total aspartic acid values (*5*) averaged 7.21 (7.20, 7.31, 7.15, 7.20, 7.17) percent. Corresponding values for nonracemic aspartic acid (*6*) in each hydrolyzate were calculated from these data, and the logarithms of the resulting values were plotted against time (Fig. 1), revealing the linear relationship that would be expected for racemization at a constant rate. The straight line shown in Fig. 1 is that fitting the data most closely according to the theory of least squares. It extrapolates at zero time to a value corresponding to 7.37 percent nonracemic aspartic acid, a value that agrees within experimental error with the mean value for total aspartic acid

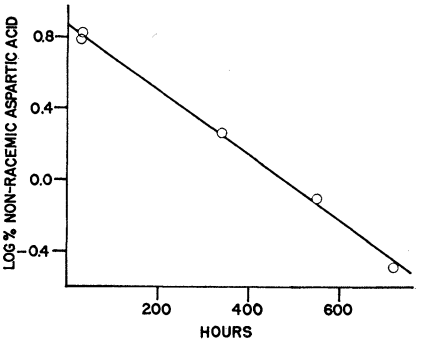

Fig. 1. Nonracemic aspartic acid in casein hydrolyzate as a function of refluxing time.

(7.21 percent). It appears, therefore, that none of the aspartic acid in casein is initially racemic and that racemization of the aspartic acid in casein proceeds essentially at the same rate before and after liberation by acid hydrolysis.

Interpolation on the curve shown in Fig. 1 indicates that the aspartic acid in mixtures of casein and 6*N* hydrochloric acid is 4.1, 8.0, and 11.8 percent racemic, respectively, after 10, 20, and 30 hours of heating the mixtures under reflux.

MERRILL N. CAMIEN
MAX S. DUNN

Chemical Laboratory,
University of California, Los Angeles

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2. Mixtures of L- and D-aspartic acids are considered here (for convenience of mathematical treatment) to be mixtures of racemic (the fraction containing equal amounts of L- and D-forms) and nonracemic (the remaining fraction) aspartic acids. It may be found algebraically that the racemic fraction is equal to twice the difference between the total and L-aspartic acids and that the nonracemic fraction equals the difference between the total and twice the L-aspartic acid.
3. This investigation (No. 115) was aided by grants from the Eli Lilly Company, the Nutrition Foundation, Swift and Company, the National Multiple Sclerosis Society, and the University of California. We are indebted to Evelyn Brown and Arthur Yuwiler for technical assistance.
4. Prepared by L. E. McClure essentially according to the procedure described by Dunn *et al.* [*J. Biol. Chem.* 155, 591 (1944)]. The product contained 14.13 percent nitrogen, 10.19 percent moisture, and 1.66 percent ash.
5. Calculated as percentage of moisture and ash-free material.
6. The differences between 7.21 (the mean total aspartic acid value) and twice the L-aspartic acid values.

27 September 1956

Interaction of Hormonal Effects: Influence of Triiodothyronine on Androgen Metabolism

It has been found (*1*) that triiodothyronine can markedly alter the relative proportion of urinary androsterone and etiocholanolone produced from endogenous or exogenous precursors without any change in the total amount of these two steroid metabolites. In Table 1 the endogenous production of androsterone and etiocholanolone in two patients during a control period without treatment is compared with that found during administration of 200 μg per day of triiodothyronine. It is evident that the thyroid hormone caused an increase in the amount of androsterone with a concomitant fall in the amount of etiocholanolone. Examination of an untreated myxedematous patient showed that etiocholanolone in the urine was 7 times higher than androsterone, despite a low level of production of these metabolites.

With this evidence that elevation of thyroid hormone increased the formation of androsterone, while a diminished thyroid secretion favored the production of etiocholanolone, it was desirable to study the influence of triiodothyronine on the metabolic products produced from

Table 1. Change in proportion of endogenous steroid metabolites with triiodothyronine. Subject P, surgical diagnosis of Stein-Leventhal syndrome; subject F, multiple sclerosis, steroid production and metabolism normal in all respects, from repeated studies in these laboratories. The dose of triiodothyronine was 200 μg per day for a period of 7 (P) and 9 days (F) prior to the studies. The methods used for steroid isolation and analysis have been described (*3*).

Subject	Treatment	Androsterone (mg/24 hr)	Etiocholanolone (mg/24 hr)
P – ♀	Control	3.8	3.9
	T – 3	5.3	2.3
F – ♂	Control	2.2	3.3
	T – 3	3.1	2.7

Table 2. Change in proprotion of metabolites of testosterone-4-C^{14} with triiodothyronine. Subject F, see Table 1; subject M, multiple sclerosis, otherwise in good health.

Subject	Treatment	Androsterone (%)*	Etiocholanolone (%)*
F – ♂	Control	37	44
	T – 3	53	29
M – ♀	Control	21	59
	T – 3	59	29

* Percentage recovered of the total radioactivity in the neutral steroid fraction of the urine, first 24 hours after hormone injection, after hydrolysis of conjugates with β-glucuronidase (Ketodase). The amount of each steroid present was measured by addition of weighed amounts of carrier, purification to radiochemical homogeneity, and calculation from the total radioactivity present in the extract and in the pure steroid. Details of isolation, purification, and measurement were essentially similar to those previously described (*2*).