fect is clear-cut. The plasma used was purposely not diluted by the recalcification technique used[7].

Synthetic organic compounds which exhibit both fibrinolytic and anticoagulant activity are bound to have therapeutic potentialities and call for further investigation. Their mechanism of action is unknown except to say that it cannot be demonstrated that the compounds directly activate purified human plasminogen, and yet an activation of the fibrinolytic system of human plasma occurs. The compounds are poorly active or non-active in bovine, cat, dog, rabbit, or rat plasma. As a working hypothesis, it is assumed that the fibrinolysis-inducing synthetic compounds inhibit (or neutralize) an antiactivator of the plasminogen system. They are furthermore capable of inhibiting the clotting process at a yet undetermined level[8].

Table II. Anticoagulant and clot-dissolving properties of *o*-thymotic acid sodium salt

mg compound per ml plasma	Mol	Recalcification time (min)	Clot dissolution after 24 h incubation
4.3	0.02	65	complete
3.87	0.018	19	complete
3.44	0.016	10	complete
3.01	0.014	6	complete
2.58	0.012	4.30	partial
2.15	0.1	4	none
1.72	0.008	4	none

Zusammenfassung. Derivate von Salicylsäure aktivieren im Reagenzglas das fibrinolytische System des menschlichen Plasmas und üben eine gerinnungshemmende Wirkung aus. Durch planmässige Veränderungen der Substituenten des Salicylsäuremoleküls gelang es, in zunehmendem Masse Verbindungen mit steigender fibrinolytischer Aktivität aufzufinden. Die gerinnungshemmende und fibrinolytische Wirkung wird am Beispiel der *ortho*-Thymotinsäure erläutert.

K. N. VON KAULLA

Department of Medicine, University of Colorado School of Medicine, Denver (Colorado USA), March 22, 1965.

[7] K. N. VON KAULLA, J. Thorac. Surg. *36*, 857 (1958).

[8] The work reported here was supported by the American Heart Association and in part by the Idaho and the Wyoming Heart Associations.

Cardiac Mitochondrial $NADH_2$-Cytochrome c Reductase and Cytochrome Oxidase after Cardiopulmonary By-Pass in Dog[1]

During and after extracorporeal circulation procedures, cardiac mitochondrial succinate dehydrogenase activity[2], and oxygen consumption rate measured by the differences between coronary arterial and venous oxygen content of blood[3] are decreased. Also, certain changes in the physicochemical properties of the actin-myosin system are observed following heart-lung by-pass procedures[4] in the dog. However, the possibilities of metabolic derangements in cardiac sarcosomes are numerous[5]. Thus, it is necessary to investigate the possible changes of mitochondrial function at the NAD-$NADH_2$ to cytochrome c, and at cytochrome c to oxygen. The cardiac mitochondrial $NADH_2$-cytochrome c reductase and cytochrome oxidase activities are studied[6,7] after partial heart-lung by-pass.

Methods. A method of cardiac mitochondrial extraction is reported elsewhere[8]. The details of isovolemic and normothermic partial cardiopulmonary by-pass perfusion procedures have also been reported previously[4]. A total of 15 dogs of both sexes, weighing 14 to 20 kg each, are anesthetized with chloralose (80 mg/kg). Of these, 10 dogs are used as the experimental group. Cardiac mitochondrial cytochrome oxidase is measured according to a modified method of SMITH[7]. The rate of oxidation of $NADH_2$ by cytochrome c (probably mediated by the $NADH_2$-cytochrome c reductase of MAHLER et al.[6]) is studied by a macro-oxygen electrode (Clark type), a specially designed magnetic stirring reaction chamber contained within a temperature-controlled water bath (a total capacity of 300 ml), and recorded on a Beckman 160 Physiological Gas Analyzer (Palo Alto, Calif. USA). The water in the bath is circulated by a Haake thermoregulator and pump. The reaction chamber contains $0.05\,M$ phosphate buffer, pH 7.4, $2 \cdot 10^{-5}\,M$ cytochrome c, and $1.2 \cdot 10^{-3}\,M$ $NADH_2$ in a final volume of 4.4 ml, equilibrated for 10 min at 25°C. The reaction is started by adding 0.1 ml of mitochondrial suspension (protein concentration of 2.08 to 3.4 mg per ml, pH 7.4, in $0.17\,M$ phosphate buffer and $0.25\,M$ sucrose solutions), and the oxidation rate of $NADH_2$ is measured for 10 min[9].

Results and discussion. The present study suggests that the cardiac mitochondrial cytochrome oxidase and the rate of $NADH_2$ oxidation by cytochrome c (probably

[1] This study was supported by Research Grant HE-09016 from the National Institutes of Health, U.S. Public Health Service.

[2] Y. W. CHO and P. M. GALLETTI, Abstract, Seventh Inter-American Congress of Cardiology, Montreal (1964), p. 236.

[3] C. DENNIS, D. P. HALL, J. R. MORENO, and Å. SENNING, Circulation Res. *10*, 298 (1962).

[4] Y. W. CHO and P. M. GALLETTI, J. thorac. cardiovasc. Surg. *47*, 628 (1964).

[5] T. P. SINGER, Ann. N.Y. Acad. Sci. *72*, 480 (1959).

[6] H. R. MAHLER, in *Methods of Enzymology* (Ed., S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 2, p. 688.

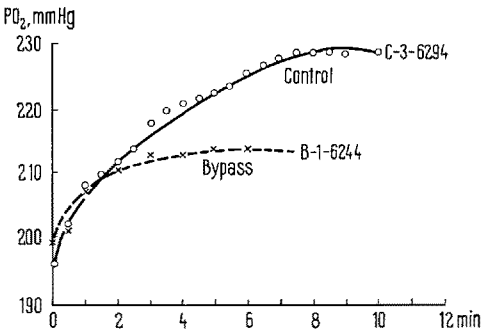
[7] L. SMITH, in *Methods of Enzymology* (Ed., S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 2, p. 735.

[8] Y. W. CHO, J. THEOGARAJ, M. M. AVIADO JR., and S. BELLET, Arch. int. Pharmacodyn. *155*, 225 (1965).

[9] E. G. BALL and O. COOPER, Proc. Nat. Acad. Sci. *43*, 357 (1957).

The oxidation rate of DPNH and cytochrome oxidase activity (Mean ± S.D.)

Experiment	Oxidation rate of DPNH (μM O_2/ml/10 min)	Cytochrome oxidase
Control	6.40 ± 2.102	46.91 ± 0.459
By-pass	3.47 ± 0.667	18.57 ± 6.320
'p' (control and by-pass)	$p < 0.02$	$p < 0.001$



The oxidation of DPNH (or $NADH_2$)[12]. Th pO_2-reaction chamber contains $2 \cdot 10^{-5} M$ cytochrome c, $1.2 \cdot 10^{-3} M$ DPNH, and $0.05 M$ phosphate buffer, pH 7.4, in a final volume of 4.4 ml. After 10 min, 0.1 ml of mitochondria (2.08 to 3.4 mg per ml) is added, and pO_2 changes are recorded by a Beckman 160 Physiological Gas Analyzer and Recorder, at 25°C for 10 min.

mediated by $NADH_2$-cytochrome c reductase) are decreased after the partial extracorporeal circulation for 3 h. The exact mechanism of mitochondrial oxidation and cytochrome oxidation are not known; however, in all probability, this cardiac mitochondrial function may be involved with MAHLER et al.'s $NADH_2$-cytochrome c reductase system[6], and probably the mitochondrial cytochrome oxidase[10], respectively. Thus, it may be concluded that all the cardiac respiratory enzymes, e.g. succinate dehydrogenase, $NADH_2$-cytochrome c reductase, and cytochrome oxidase, may be depressed by cardiopulmonary by-pass procedures[11]. Also, our earlier study suggests that the mitochondrion has lost its ability to control respiration in the presence of glucose and hexokinase after the perfusion procedures[11].

Zusammenfassung. Nach kurzfristiger extrakorporaler Zirkulation beim Hund wurden Cytochrom-Oxydase-Aktivität und $NADH_2$-Oxydationsrate in den Mitochondrien des Herzmuskels im Vergleich zu Kontrolltieren vermindert gefunden.

Y. W. CHO

Research Physiology Section, Division of Cardiology, Philadelphia General Hospital; and Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia (Pennsylvania USA), March 30, 1965.

[10] O. HAYAISHI, Ann. Rev. Biochem. *31*, 25 (1962).
[11] Y. W. CHO, Angiology, in press.
[12] $NADH_2$ (nicotineamide-adenine dinucleotide, reduced) was formerly DPNH (diphosphopyridine nucleotide, reduced) (1962).

Immunological Response Between Protozoa Symbiotic to a Roach and a Termite

Immunological reactions and biochemical studies have proved to be valuable criteria for showing resemblances in strains or species of Protozoa. Specific methods have been employed by NOGUCHI[1,2], TALIAFERRO[3,4], BERNHEIMER and HARRISON[5], SOLTYS[6], SONNEBORN[7], SEED[8], and most recently by SAMUELS[9], to demonstrate the validity of proposed species (sometimes strains) of *Paramecium, Leishmania, Trypanosoma,* and *Trichomonas* where morphological characters are morphologically nonspecific in different species of the genus.

According to CLEVELAND[10] at least 7 families, 14 genera, and more than 30 species of flagellate protozoa inhabit the hind-gut of the wood-feeding roach *Cryptocercus punctulatus*. These flagellates have been reported by CLEVELAND[10] to be closely related to those of termites, some being species of genera and others genera of families living in *Cryptocercus*. Other flagellate genera of termite species, however, are not immediately recognizable as having a *Cryptocercus* representative. The question then arises, whether species of flagellate protozoa, e.g. *Trichonympha* in *Cryptocercus*, and flagellate protozoa, e.g. *Trichonympha*, found in termites, in spite of acceptable and strong morphological resemblances, in fact, arose independently in their similar environments, or were carried over from a progenitor common to both.

Various implications are found in the literature which suggest that roaches and termites are off-shoots of the primitive group, the Protoblattoidae (IMMS[11] and HOLMGREN[12]), or that termites are an off-shoot from roaches (CLEVELAND[10]).

It is impossible at present to make a detailed comparison of the protozoa of *Cryptocercus* with those from

[1] H. NOGUCHI, Proc. Intern. Conf. Health Problems, Kingston, B.W.I. (1924).
[2] H. NOGUCHI, J. exp. Med. *44*, 327 (1926).
[3] W. H. TALIAFERRO, *Immunology of Parasitic Infections* (New York 1929).
[4] W. H. TALIAFERRO, in *Protozoa in Biological Research* (Ed., G. N. CALKINS and F. SUMMERS; Columbia Press, New York 1941).
[5] A. W. BERNHEIMER and J. A. HARRISON, J. Immun. *39*, 73 (1940).
[6] M. A. SOLTYS, J. Parasit. *47*, 390 (1957).
[7] T. M. SONNEBORN, Am. Assoc. Advan. Sci. Symp., Washington, D.C. (1957).
[8] J. R. SEED, J. Protozool. *10* (4), 380 (1963).
[9] R. SAMUELS and H. CHUN-HOON, J. Protozool. *11* (1), 36 (1964).
[10] L. R. CLEVELAND and S. R. HALL, Mem. Am. Acad. Arts Sci. *17*, 185 (1934).
[11] A. D. IMMS, Phil. Trans. R. Soc. Series B, *209*, 75 (1919).
[12] N. HOLMGREN, K. svenska Vetensk.-Akad. Handl. *44*, 1 (1909).