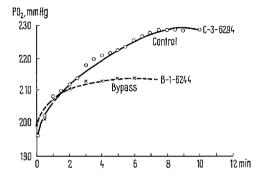
The oxidation rate of DPNH and cytochrome oxidase activity (Mean \pm S.D.)

Experiment	Oxidation rate of DPNH $(\mu M O_2/\text{ml}/10 \text{ min})$	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 NADH₂)¹². Th pO₂-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 pO₂ changes are recorded by a Beckman 160 Physiological Gas Analyzer and Recorder, at 25°C for 10 min.

mediated by NADH₂-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 NADH₂-cytochrome c reductase system⁶, and probably the mitochondrial cytochrome oxidase ¹⁰, respectively. Thus, it may be concluded that all the cardiac respiratory enzymes, e.g. succinate dehydrogenase, NADH₂-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 NADH₂-Oxydationsrate in den Mitochondrien des Herzmuskels im Vergleich zu Kontrolltieren vermindert gefunden.

Y, W. Сно

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 NADH₂ (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 Harrison⁵, Soltys⁶, Sonneborn⁷, Seed⁸, and most recently by Samuels⁹, 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.

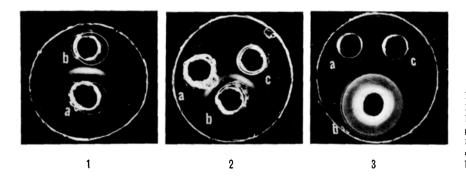
According to CLEVELAND ¹⁰ at least 7 families, 14 genera, and more than 30 species of flagellate protozoa inhabit the hind-gut of the wood-feeding roach *Cryptocercus punctulatus*. These flagellates have been reported by CLEVELAND ¹⁰ to be closely related to those of termites, some being species of genera 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 in-

dependently 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 (IMMS¹¹ and HOLMGREN¹²), or that termites are an off-shoot from roaches (CLEVELAND¹⁰).

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. Tallaferro, 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,
- ¹¹ A. D. Imms, Phil. Trans. R. Soc. Series B, 209, 75 (1919).
- 12 N. Holmgren, K. svenska Vetensk.-Akad. Handl. 44, 1 (1909).



Photographs of Ouchterlony plates (after 18 day course of immunization). – Fig. 1. Depot a, containing roach-protozoa antigen; b, anti-roach rabbit serum. – Fig. 2. Same as in Figure 1, only c depot added containing termite-protozoa antigen. – Fig. 3. Control.

known termites, because many of the protozoa of termites have not been adequately identified (CLEVELAND 10). Thus, some other means must be employed to attempt to show this relationship (if there exists such a relationship).

This study was undertaken in an attempt to establish a method whereby immunological responses between protozoa symbiotic to a roach and a termite might be employed to provide chemical evidence to support existing morphological evidence for protozoan relationship in the dissimilar hosts.

As part of this investigation, animal sensitization to hind-gut protozoa of the roach Cryptocercus was conducted in rabbits according to 'standard immunological protocol'. Anti-roach rabbit serum resulted in interfacial ring and flocculation tests indicating an antibody titer of 1/1024. Results of agar diffusion according to Ouchterlony (Figures 1-3) indicated a similar antibody response between protozoa symbiotic to the roach Cryptocercus and the termite Zootermopsis. Subsequent agglutination tests revealed individual clumping of two different genera of protozoa (Trichonympha and Monocercomonoides) of which Trichonympha is common to both roach and ter-

mite. Results at present indicate close chemical affinity between protozoa of the roach *Cryptocercus punctulatus* and the termite *Zootermopsis nevadensis* ¹³.

Résumé. L'analyse des responsabilités immunologiques après immunisation d'animaux expérimentaux avec les protozoaires symbiotiques du 'roach', Cryptocercus et du termite Zootermopsis indique une étroite affinité chimique entre les protozoas de ces hôtes. Les résultats semblent confirmer indirectement la taxonomie morphologique courante des hôtes.

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13 Grateful acknowledgment is made to Dr. H. RITTER JR. for guidance during the course of this investigation.

Aspermatogenic Antigen from Brain

It has been known for many years that brain and testicle share some antigenic similarities in that antisera to these two organs cross-react¹. The nature of the common antigen(s) is unknown. It has been reported that homologous brain homogenate can induce testicular damage in guinea-pigs². Since homologous testicular homogenate also causes testicular lesions, the inference is that antigenic similarity of the brain and testicle includes an aspermatogenic factor (ASF). However, inasmuch as injection of brain homogenate can induce acute disseminating encephalomyelitis, the testicular dyscrasia observed in such animals could result from a general debilitating condition. The current experiments were designed to determine the immunologic specificity of the brain antigen causing testicular damage.

The experimental design is indicated in the Table. The general procedure for preparing the adjuvant-antigen emulsions and the injection routine have been described in detail elsewhere ^{3,4}. The extraction methods that have been applied to testes ⁵ were also applied to brain. Brains were obtained from 50 exsanguinated male and 50 exsanguinated female guinea-pigs. The brains were kept at

— 20°C until used. For further purification of the extracted antigens, modifications of the extraction were employed: these included re-precipitation with trichloracetic acid, extensive dialysis against running tap water (72 h), and partitioning between chloroform and butanol using the aqueous phase which was lyophilized after further dialysis.

From the Table, it can be seen that brain extracts were potent in inducing depletion of spermatogenic tissue as indicated by the testicular damage rating in which increasing numerals (+1 to + 4) denote increased damage⁴. A curious finding which requires further work for substantiation was that extract of female brain was more potent in inducing hypospermatogenesis or aspermatogenesis than was the extract of male brain.

In view of these results in which testicular damage was elicited by brain extracts without accompanying neuro-

¹ J. H. Lewis, J. Immunol. 27, 473 (1934).

² S. Katsh and D. W. Bishop, J. Embryol. exp. Morph. 6, 94 (1958).

³ S. Katsh, Int. Arch. Allergy 15, 172 (1959).

⁴ S. Katsh, Int. Arch. Allergy 24, 319 (1964).

⁵ S. Katsh, Int. Arch. Allergy 16, 241 (1960).