true, non-colloidal solution. Within the experimental accuracy, the molecular weight was independent of the extraction method employed as well as time of exposure to $C^*O_2$.

The reversible uptake of $CO_2$ in the dark may be written as follows:

$$(I) \qquad CO_2 + RH \rightleftarrows R{-}\overset{\overset{O}{\|}}{C}{-}OH.$$

The formation of carboxyl is a probable reaction energetically and structurally and is suggested by the presence of radioactivity in the carboxyl group. According to thermal data for similar reactions, this process may be exothermic by 1–2 K. cal. The experimental data accumulated thus far indicate $\triangle F$ can be unfavorable by 1–2 K. cal. Free energy values for a reaction such as I, involving large molecules, are not available, however. For small molecules, the free energy change is +5–10 K. cal. Conjugation in the "R" to the carboxyl group may furnish resonance energy to help the reaction. A more favorable free energy balance may be gained by the occurrence simultaneously of processes involving other molecules or taking place within R itself. This reaction is undoubtedly catalyzed by an enzyme system. Evidence bearing on the nature of RCOOH (at least after extraction) is its molecular weight of 3,000 or more, solubility in water, insolubility in ether, absence of free carbonyl groups, presence of alcoholic groups and absence of pigments (chlorophyll, etc.).

At present the dark reversible uptake of $CO_2$ may reasonably be considered a part of the photosynthetic process,[8] since (1) it is found to be similarly poisoned by HCN, (2) the molecular weight of R is the same as that of the photosynthetic products (whether ultimate or not) formed in the light. That $CO_2$ does not combine with chlorophyll but with some other substance present in the cell has been suggested by many workers.[9]

Since the net reaction for photosynthesis is

$$nh\nu + H_2O + CO_2 \longrightarrow 1/x(C \cdot H_2O)_x + O_2$$

one may, without specifying the various steps[10] involved, represent the photochemical process by the following schematic equation:

$$(II) \qquad RCOOH + H_2O + nh\nu \longrightarrow RCH_2OH + O_2.$$

The $CO$–$O_2$ balance in photosynthesis, together with the absence of carbonyl groups in the observed products, infers reduction to hydroxyl. The similarity between the molecular weights and other properties of the molecules in the light and dark reactions suggests strongly that R in I and II are identical. The energy required for II is about 110 K. cal per mole of $CO_2$ reduced.

We may add that formaldehyde formation, sometimes postulated, would require about 135 K. cal per mole. In addition the above picture avoids the necessity of polymerization steps. The $RCH_2OH$ may add another $CO_2$ and repeat the cycle to build up long carbohydrate-like chains. Indeed, R itself may be such a chain. Processes in which carbohydrates are formed by interaction of accumulated reduction products, are of course, not excluded.

We wish to acknowledge our indebtedness to our many associates, and especially to Professors W. M. Latimer, W. F. Libby, W. C. Bray and T. D. Stewart, for their interest and many valuable discussions and suggestions. The continued cooperation of Professor E. O. Lawrence and his colleagues in the Radiation Laboratory has made this work possible. Our thanks are also due to the Rockefeller Foundation for financial support to the Radiation Laboratory.

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[8] We wish to thank Professor G. Mackinney and Dr. H. A. Barker for friendly criticism.

[9] R. Emerson, *Ergeb. Enzymforsch.*, 5: 305, 1936; H. Gaffron and K. Wohl, *Naturwissenschaften*, 24: 81, 103, 1936; K. V. Thimann, SCIENCE, 88: 50c, 1938.

[10] These may be part of a process involving either a photosynthetic unit (Emerson and Arnold, *Jour. Gen. Phys.*, 16: 191, 1932) or a sequence of reactions as suggested by Franck and Herzfeld, *Jour. Chem. Phys.*, 5: 231, 1937.

## THE PREPARATION AND PURIFICATION OF BRUCELLA ANTISERUM[1]

THE constituent of *Brucella* cells responsible for the clinical manifestations of brucellosis in man and animals appears from our studies to be a toxic soluble fraction.[2] Its toxicity may be demonstrated by any route of parental injection.

Preliminary studies have shown that the toxic action of the soluble fraction for experimental animals can be neutralized *in vitro* before injection by the precipitating action of a specific antiserum or by injecting a sufficient amount of antiserum shortly before the toxic antigen.

In order to obtain an antiserum of high precipitin titer or neutralizing power, and as free as possible of non-specific substance for therapeutic application in certain forms of human brucellosis, several procedures were examined.

The serum used was obtained from the cow. The fresh untreated serum precipitated the antigenic frac-

[1] Journal Article No. 396 n.s. from the Michigan Agricultural Experiment Station. This study was supported by a grant from the Horace H. and Mary A. Rackham Fund.

[2] R. B. Pennell and I. F. Huddleson, *Technical Bulletin* 156, Michigan Agricultural Experiment Station, 1937.

tion up to a dilution of 1:32,000. When the serum was diluted progressively, and the dilution of the antigen kept constant, precipitation ceased above a 1:40 dilution.

The procedures examined for the concentration and purification of the antiserum were: (1) dissociation of specific precipitates; (2) precipitation of immune globulin by dilution of serum with distilled water; (3) precipitation of immune globulin with alcohol; (4) purification by the use of salts of heavy metals; (5) disaggregation of serum proteins by means of pepsin and removal of non-specific protein by heat coagulation; (6) dissociation of serum proteins by sodium-chloride.

A combination of procedures (4) and (5) was the only one that proved to be of any value. Procedure (5) used by us is a modification of the ones described by Parfentjev (2) and Pope (1), (2), (3).

An outline of the procedure found adaptable to the purification of *Brucella* antiserum from the cow is as follows: To 500 cc of fresh serum was added 446 cc of 0.5 per cent. $AlCl_3$. The precipitate was centrifuged out and the excess of $AlCl_3$ removed from the supernatant by neutralization. The volume of supernatant recovered was 700 cc. Following this step, purified pepsin (Difco) was added to the serum to a final concentration of 0.5 per cent., the pH adjusted to 4 with citric acid, and the mixture digested for 1 hour at 37° C. Following digestion, the mixture was adjusted to a pH of 5.5, heated for 30 minutes at 60° C., and then centrifuged to remove heat-coagulated protein. The supernatant was recovered and precipitated with $(NH_4)_2SO_4$ at half saturation. The precipitate formed was removed and dialyzed in a Cellophane casing under pressure against distilled water until free from $(NH_4)_2SO_4$. The volume of the dialysate was now 300 cc.

Methiolate was added as a preservative and sufficient sodium chloride added to make the final concentration 0.85 per cent. The purified serum was sterilized by passing through a Seitz filter.

Table I shows the precipitation valves of the serum at different steps in its preparation.

The results of one of several experiments which demonstrate the high neutralizing ability of the purified antiserum *in vivo* are illustrated in Table II. In order to obtain complete neutralization of the toxic antigen *in vivo*, it is necessary to inject the antiserum at least ten minutes before the antigen. If a toxic dose of the antigen is injected before the antiserum or the two are injected simultaneously, no neutralization is obtained.

In order to determine whether the purified antiserum had any therapeutic action on the course of certain forms of human brucellosis, five patients were given daily subcutaneous injections of the purified serum in amounts varying from 0.1 cc to 5 cc. Two of the patients failed to respond to treatment with the antiserum. The temperatures of two returned to normal and remained so after two injections. The temperature of the remaining one returned to normal following one injection, but again became elevated after a lapse of seven days. Three more daily injections were then given following the rise in temperature. The temperature again returned to normal and remained so.

TABLE I

| | Maximum precipitation of antigen | Maximum precipitin titer |
|---|---|---|
| Untreated serum ..... | 1:32,000 | 1:40 |
| After $AlCl_3$ treatment... | 1:256,000 | 1:128 |
| After pepsin treatment.. | 1:4,000,000 | 1:2048 |

TABLE II

NEUTRALIZING ACTION OF PURIFIED *Brucella* ANTISERUM ON *Brucella* ENDOANTIGEN *in vivo*

| G. Pig No. | Amount of anti-serum cc | Amount of endo-antigen mg | Tempera-ture reaction | Result |
|---|---|---|---|---|
| 1 .... | 1 | 10 | Normal | Lived |
| 2 .... | 0.5 | " | " | " |
| 3 .... | 0.1 | " | " | " |
| 4 .... | 0.05 | " | " | " |
| 5 .... | 0.01 | " | " | " |
| 6 .... | 0.005 | " | " | " |
| 7 .... | 0.0025 | " | " | " |
| 8 .... | 0.00125 | " | " | " |
| 9 .... | 0.000625 | " | −5 degrees | Dead 6 hours |
| 10 .... | 0.000312 | " | Normal | Lived |
| 11 .... | 0.000156 | " | " | " |
| 12 .... | None | " | −5 degrees | Dead 6 hours |
| 13 .... | " | " | −5 degrees | " |

All injections intradermally.
Antiserum given one hour before endoantigen.

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## SELENIUM AND DUCK SICKNESS

A PREVIOUS communication[1] indicated that low concentrations of selenium produced poisoning in ducks in which the syndrome was identical with that described as produced by *Clostridium botulinum* type C.[2] Since the original work it has been possible to secure field material which would indicate that selenium is an important factor in western duck sickness in the areas studied. These results appear sufficiently significant to warrant recording.

Analyses of the livers of ducks and shore birds found dying of duck sickness in the Great Bear Marsh area, Utah Lake and the Lake Front Project, fifteen miles northwest of Salt Lake City, showed definite traces of selenium. Black ducks and mallards collected in the vicinity of Pymatuning Swamp, Pennsylvania (a non-seleniferous area), exhibited negative tests when

[1] A. C. Twomey and S. J. Twomey, SCIENCE, 83: 470, 1936.
[2] U. S. Dept. Agr., *Bull.* 411, May, 1934.