

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 $(\text{NH}_4)_2\text{SO}_4$ at half saturation. The precipitate formed was removed and dialyzed in a Cellophane casing under pressure against distilled water until free from $(\text{NH}_4)_2\text{SO}_4$. 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 values 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

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	Temperature 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.

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.

I. FOREST HUDDLESON

R. B. PENNELL

MICHIGAN STATE COLLEGE

SELENIUM AND DUCK SICKNESS

A PREVIOUS communication¹ 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.

treated in like manner. In the chemical analyses of these tissues, a modification of the Robinson, Williams,

TABLE 1
LIVER ANALYSES

No.	Species	Dry wt. liver	Ppm selenium
A*1	American pintail	5.197 gms	7
2	" "	4.201	16
3	Common mallard	3.667	19
4	" "	6.776	16
5	" "	4.716	40
6	Green-winged teal	1.669	44
7	" "	1.426	52
8	" "	1.307	76
9	" "	2.311	43
10	White-faced glossy ibis	4.075	25
11	Avocet	2.971	25
B†1	American pintail	3.196	11
2	" "	3.268	18
3	" "	2.065	39
4	" "	3.358	24
5	" "	1.519	40
6	Common mallard	2.369	34
7	Green-winged teal	1.635	148
8	" "	1.629	49
9	" "	2.522	32
10	" "	1.300	92
11	Shoveller	3.105	19
12	American coot	2.434	49
C‡1	Green-winged teal	2.335	none
2	Redhead	5.065	none
3	American pintail	5.380	none
4	" "	4.635	none
5	Common mallard	6.015	10
6	Redhead	5.910	10
7	Common mallard (juv.)	4.545	10
D§1	Common mallard	6.737	none
2	" "	6.091	none
3	" "	12.477	none
4	" "	7.976	none
5	" "	6.537	none
6	Common black duck	7.326	none
7	" "	8.626	none

* A Samples from Utah Lake (dead ducks).

† B Samples from Lake Front Project, 15 miles northwest of Salt Lake City (dead ducks).

‡ C Samples from supposedly healthy birds collected on shooting grounds near Brigham, Utah, prior to an outbreak.

§ D Samples from healthy birds obtained from Pymatuning Swamp area, Pennsylvania.

Dudley and Byer^{3,4} method of determining selenium was used. Blank tests were run on all reagents. (See Table 1.)

A marked decrease in the size and weight of livers was noted in the specimens obtained from waterfowl dying of the duck sickness. This supports the experimental finding that ducks, which were poisoned on low concentrations of selenium as sodium selenite (up to 18 ppm.), fed in the drinking water, showed a reduction in the size and weight of the livers. The experimental birds also showed all the symptoms of western duck sickness in successive stages. Higher concentrations of selenium produced death within ten to twenty-four hours, and the birds died without perceptible shrinkage of livers.

Vegetation, including *Potamogeton pectinatus*, *Salicornia rubra*, *Chara* and *Ruppia maritima*, taken from Willard Spur and Unit 3 of the Great Bear Marsh area on August 29, 1939, showed no evidence for the presence of selenium. Soil and water samples taken from the same places contained no selenium.

Although large numbers of birds die during major outbreaks of duck sickness, they represent but a small percentage of the actual birds present in the areas.

The waterfowl poisoned with selenium probably ingest it in either an organic or inorganic form from small, restricted areas.

These results would indicate that selenium is a factor in western duck sickness.

ARTHUR C. TWOMEY
SARAH J. TWOMEY

CARNEGIE MUSEUM

LORING R. WILLIAMS

UNIVERSITY OF NEVADA

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD FOR THE FILING OF MICROFILM RECORDS IN SHORT LENGTH STRIPS

RECENTLY L. R. Dice¹ described a simple method of filing microfilm records. For the past three years, we have employed a different method which we believe has advantages commending its description. We have found the method applicable to an industrial laboratory library, a school library and to private libraries.

Much attention has been given to the filing of 25-, 50- and 100-foot lengths of microfilm copies of documentary material. Little attention has been given to lengths of film of not over 3 feet, and often under 18 inches in length. As it is customary to photograph 16 pages of the ordinary magazine, journal or similar material on a 1-foot length of 35-millimeter film, it often happens, particularly in the fields of science, that an entire article can be copied on one foot or less

of film. We have found it more convenient to use such copies in their short-strip form rather than splice them together into long film lengths.

The film strips are conveniently stored in an ordinary 10×12-inch filing cabinet drawer by making use of specially prepared filing cards of this size. These cards are prepared by taking a 10×12-inch piece of 1/16-inch cardboard and sewing onto one or both surfaces of the card layers of cloth, so that pockets are formed. A convenient type of card, which we have employed extensively, consists of four pieces of cloth sewn on one side of the cardboard. This results in six rows of three pockets each or a total of eighteen pockets. Each pocket is capable of holding a one-foot film strip, or 16 pages; thus, on one card, it is possible to file 288 pages of microfilm copies of documentary material.

³ Robinson, *et al.*, *Ind. Eng. Chem. Anal. Ed.*, 6: 274, 1934.

⁴ *South Dakota Exp. Sta. Bull.*, No. 311, 1937.

¹ SCIENCE, 89: 39-40, 1939.