The mechanism naturally had to get around the fact that as liquid flows from a container the head is diminished and consequently the rate of flow decreased.

The use of a float valve in keeping a constant head was not satisfactory, so the siphon principle was employed and later modified to the form described as follows:

(1) Secure a container (A) of fairly light material which is large enough to hold the liquid that is being used.

(2) Fashion some sort of float which will rest on the surface of the liquid in the container. It must be a flat float which will not turn over.

(3) Run the short arm of a U-shaped siphon tube through the center of the float so that one end of the tube is submerged and the other (lower) end is outside the container. The height of the U must be great enough so that as the liquid is siphoned off and the float sinks the tube will not strike the lip of the container.

(4) The arrangement above will give a fairly even flow, providing the depth of the liquid is not great, but in order to give an unvarying flow the container of liquid (A) must be floated in a second container (B), so that as the liquid is siphoned from (A) it decreases the weight of (A) and causes it to rise in the water in container (B). Thus the two ends of the siphon are kept at exactly the same level throughout the process.

The apparatus has been described very roughly, and it is necessary to watch the balance and points of contact so that the rising and dropping of the floating bodies is not hindered.

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# SPECIAL ARTICLES

## LATENT PSITTACOSIS AND SALMONELLA PSITTACOSIS INFECTION IN SOUTH AMERICAN PARROTLETS AND CONURES[1]

DURING the month of May, 1933, the United States Quarantine Station at Angel Island received a shipment of tropical birds for the customary isolation of 2 weeks. The parrotlets, paroquets and conures had been caught in 1932 during the month of October 300 miles south from Barranquilla. They were held at the establishment of a dealer at Magangue and then at Port Colombia, from where they were shipped on April 20, 1933. Through the courtesy of Dr. H. A. Spencer, medical officer in charge of the United States Public Health Quarantine Station, 52 of the psittacine birds, which died during the quarantine period from May 11 to June 13, were sent to the laboratory for study. Complete autopsies, cultural examinations and mouse inoculations with the organ suspensions were made on every bird. Since the spleens and livers of 2 spectacled parrotlets produced in mice sterile lesions suggestive of psittacosis with positive findings of L. C. L. bodies, the State Department of Public Health arranged with the United States Public

[1] From the George Williams Hooper Foundation, University of California, San Francisco, California.

TABLE I

| | No. | Sex | | Splenic tumor and liver necroses | Psittacosis virus | Salmonella "aertryck" |
|---|---|---|---|---|---|---|
| | | M. | F. | | | |
| Tovi paroquet, *Brotogeris jugularis** (Müller) | 39 | 15 | 24 | 4 | — | 4 |
| Spectacled parrotlet, *Psittacula conspicillata* (Lafresnaye) | 37 | 26 | 11 | 4 | 2 | 4 |
| Spengel parrotlet, *Psittacula spengeli* (Hartlaub) | 16 | 9 | 7 | 3 | 4 = (25 per cent.) | — |
| Petz paroquet or conure, *Eupsittula canicularis* (Linnaeus) | 29 | 12 | 17 | 15 | — | 5 |
| Brown-throated paroquet, *Eupsittula pertinax aeruginosus* (Linnaeus) | 11 | 6 | 5 | 3 | 2 | 3 |
| Totals | 132 | 68 | 64 | 29 | 8 = 6 per cent. | 16 = 12 per cent. |

* Determined by Professor J. Grinnell from Ridgway's "Birds of North and Middle America," Part VII, 1916.

Health Service and the shipper for the destruction of the paroquets and parrotlets. A total of 132 birds, including those which had succumbed during the quarantine period, has been examined. The results are summarized in Table I.

In general, the data confirm previous observations that tropical psittacine birds are not infrequently carriers of Salmonella organisms, which are related biochemically to the *S. psittacosis*. The 19 different strains, which have been isolated either directly from the enlarged spleens, livers and heart blood, or indirectly from the mice inoculated with the organs, are, serologically, closely related to the *S. aertryck* but consist of a number of diphasic variants. The parrotlets, paroquets and conures, infected with these bacteria, had either succumbed to the infection or were carriers. When the former was the case, the intestinal tube was definitely inflamed, the anus soiled with greenish material and the spleen enlarged and soft. The carriers were recognized by the atrophic pectoral muscles, the large spleen and the voluminous fatty liver diffusely studded with fine grayish necroses. Aside from these usual lesions, 2 Tovi paroquets presented bilateral pneumonic patches. Microscopically, the anatomical changes are identical with those described for avian salmonellosis by J. R. Meyer.[2] Acidophilic intranuclear inclusions characteristic of the Brazilian virus have not been observed. The Salmonella strains exhibited their usual host range of pathogenicity. They infect, with ease, shell parrakeets and ricebirds per os. If accidentally encountered by the inexperienced, the lesions in the birds may lead to confusion, although the cultural examination with brilliant green media will reveal the specific cause without difficulty.

More important is the demonstration of the psittacosis virus in the spleen and liver of 4 immature Spengels, 2 spectacled parrotlets and 2 brown-throated paroquets. Fatal infections were seen in 2 *Ps. spengeli* and 1 *Ps. conspicillata*. The cadavers were emaciated, the tail feathers soiled, the spleens slightly enlarged (3×3, 5×5 mm), the fatty livers showed a few necrotic areas (readily distinguished from the Salmonella lesions) and light grayish kidneys. A latent infection was indicated in the 5 other parrotlets and paroquets by a splenic tumor and a yellowish liver. Cultures prepared from the heart blood and organ suspensions remained sterile. Mice injected intraperitoneally with 1 cc of a broth suspension of the organs became ill in from 7 to 14 days. When death ensued, they showed the usual lesions encountered in psittacosis infections of these rodents. L. C. L. bodies were readily demonstrated in the impression preparations. The specificity of the infective agent was proven by 29 passages in 36 weeks. Its filterability and the pathogenicity for rice-birds and shell parrakeets were established by injection, by exposure and by the guinea pig skin test. Preliminary cross immunity tests indicate a close relationship, if not identity, with the California virus. It is regrettable that the actual number of latent infections within the group of 132 birds could not have been determined with certainty. However, it was impractical to filter all the organ suspensions of the parrotlets, which had lesions suggestive of psittacosis but were heavily invaded by *S. psittacosis*. Thus, the possibility must be kept in mind that mixed infections of the virus with bacteria may have existed in the flock. Filtrates of several livers, which presented lesions suggestive of old necroses, were tested on shell parrakeets in order to demonstrate the Brazilian virus of Pacheco, Bier and Meyer,[3] which is species specific and is not transferable to mice. The results were negative. Two representatives each of the 5 species of Psittacidae, belonging to the same shipment, were kept under observation for several weeks. They were then injected intramuscularly with a very active California passage virus. The parrotlets and Petz conures died within from 10 to 26 days with the lesions and L. C. L. findings of acute psittacosis, while the Tovi and brown-throated paroquets remained well despite repeated injections of virus. Susceptibility tests on a limited number of birds, as a rule, remain inconclusive. One may, therefore, merely conclude that the highly treasured Spengel's and spectacled parrotlets are quite susceptible and, consequently, may sometimes become spontaneous carriers of the psittacosis virus, particularly when they are immature. In this respect, they behave like the budgerigars. The resistance of the paroquets against an acute infection was anticipated, since the birds were mature. It is well known from previous studies and others to be reported that the adult population of a susceptible species of psittacine birds, grown or reared in an endemic area, is largely an immune one.

The observations conclusively establish the existence of psittacosis in tropical birds from Colombia and, consequently, justifies the protective measures which have been instituted against the importation of these pets. Unfortunately, the origin of the disease, whether contracted in nature or in the bird stores of Barranquilla, remains undetermined. It seems reasonable, however, in the light of other studies, to assume that avian psittacosis is widely distributed among South American parrots, parrotlets and paroquets. Furthermore, there is no doubt that in case conditions favorable for its development exist, and

[2] *Arch. Inst. Biol. São Paulo*, 1931, 4: 25, 1931.

[3] *Memonas do Instituto Oswaldo Cruz*, 26: 169, 1932. Th. M. Rivers and F. F. Schwentker, *Jour. Exper. Med.*, 55: 911, 1932.

where there are many acute fatal avian infections, the disease may be transferred to man and may be spread to other cage birds in pet shops or bird establishments.

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## POSSIBLE CHEMICAL NATURE OF TOBACCO MOSAIC VIRUS[1]

UNDER the above title Barton-Wright and McBain[2] make the interesting announcement that virus fractions have been obtained which were free of nitrogen.

Lojkin and Vinson[3] reported the inactivation of purified virus preparations by trypsin. Caldwell[4] announced that this inactivation by trypsin is only apparent; for upon heating the trypsin digest to a suitable temperature, the activity of the virus was restored. Our results during the past year fully agree with Caldwell's announcement. Since the protein-splitting enzymes do not readily attack this virus, it would seem possible that it might be non-nitrogenous in character; hence the announcement of Barton-Wright and McBain is in keeping with this possibility.

Barton-Wright and McBain precipitated the virus from juice of diseased plants with a solution of lead acetate; removed the virus from this precipitate with neutral phosphate solution, then precipitated the virus from the neutral phosphate solution with safranin. The safranin-virus precipitate was suspended in water and the safranin removed with normal amyl alcohol. The decolorized aqueous phase thus obtained was infectious and contained no nitrogen.

Under our conditions and using plants of *Nicotiana Tabacum,* var. Turkish, I still find nitrogen present in infectious preparations obtained as described above. The nitrogen content, however, is not high, especially when the diseased plants have been grown during the short gloomy days of midwinter. Also, in extracting the safranin with normal amyl alcohol, emulsions tend to form and the undecomposed safranin precipitate tends to concentrate in the surface films. As the emulsion breaks, the safranin precipitate collects at the amyl alcohol-water interface. In case this interfacial material is not allowed to remain with the aqueous phase after each extraction, the nitrogen content of the final virus fraction may be very low, indeed, and the results indicate that the infective power was also low. When, however, the leaves of 10 plants were rubbed with a cloth dipped in the virus preparation, 100 per cent. infection was produced. Starting with 500 cc of juice from diseased plants, carrying through and running the Kjeldahl determination on the entire sample (except for an aliquot of 20 cc removed for inoculating plants and for pH determinations), the final virus fraction may contain only one or two milligrams of nitrogen. When, however, care was exercised to retain the interfacial layer with the aqueous phase after each extraction until decomposition was complete and there was no further interfacial layer, the nitrogen content of the final virus fraction from 500 cc of juice was found to vary from 8 to 24 milligrams in 8 experiments. The number of plants diseased out of one hundred plants inoculated with the last mentioned preparation was much greater than in the case of inoculations with those preparations obtained by discarding the interfacial layer each time.

Vinson and Petre[5] have already reported that a suspension of the washed interfacial layer is infectious. Had nitrogen determinations been made on the ordinary sample derived from 25 cc or even 100 cc of juice, it would have been impossible to detect nitrogen in some of the above fractions by the ordinary Kjeldahl method. It is also interesting to note that when Lloyd's reagent[6] was employed to decompose the safranin precipitate the nitrogen content and infective power of the final fraction were increased. This greater infective power should not be taken, however, as an absolute indication of greater virus content, since the fraction obtained by the use of normal amyl alcohol and that obtained by the use of Lloyd's reagent passed through different procedures and hence were not comparable.

Barton-Wright and McBain also state that their fractions, obtained by the procedure described above, were free of phosphate. Again, under our conditions, and starting with 500 cc of juice from diseased Turkish tobacco plants, the final infectious virus fraction obtained by the method of Barton-Wright and McBain contained phosphorus. Phosphorus was present to the extent of about one half milligram even when the safranin precipitate had been washed 3 times with a concentration of safranin (200 cc of a 1 per cent. aqueous solution added to 500 cc of redistilled water) equal to that in the mother liquor from the safranin precipitate, then washed once with redistilled water. It would seem very difficult to obtain a safranin precipitate from such a highly concentrated

[1] Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station. Journal Series No. 380.

[2] E. Barton-Wright and A. M. McBain, "Possible Chemical Nature of Tobacco Mosaic Virus," *Nature,* 132: 1003, December 30, 1933.

[3] Mary Lojkin and C. G. Vinson, "Effect of Enzymes upon the Infectivity of the Virus of Tobacco Mosaic," *Contr. Boyce Thompson Institute,* 3: 147–162, 1931.

[4] John Caldwell. *Ann. of Appl. Biol.,* 20: 111, February, 1933.

[5] C. G. Vinson and A. W. Petre, "Mosaic Disease of Tobacco," *Contr. Boyce Thompson Institute,* 1: 479–503, 1929.

[6] C. G. Vinson, "Mosaic Disease of Tobacco. V. Decomposition of the Safranin-Virus Precipitate," *Phytopathology,* 22: 965–975, December, 1932.