

the left) was very similar to the one presented usually by secondary cultures of bovine bacilli on Petragnani as far as the evenness of the growth is concerned but was much thicker.

The study of the cultural characteristics was already sufficient to discard for such dysgonic strains the possible alternative diagnosis of bovine strains which are not inhibited in their growth by glycerin (8). In fact, the colonies appearing separately on primary cultures or so obtained on subcultures by means of a special technique were in great majority quite different from the perfectly smooth, shining, dome-shaped ones, typical of bovine strains.

All transitional grades were observed in the same strain or in different strains obtained from the same patient: at one end some colonies identical to those belonging to the typical human eugonic strains, very rough and well developed, in the middle in greater number, dysgonic and less rough ones (Photo 4), at the other end smooth bovine-like form of colony, rarely met with (Photo 5).

If single colonies of the latter form were observed by the naked eye, they would perhaps be typed as bovine ones. But when they were considered together with all the others in the same tube, one could easily perceive that they were but almost an extreme step in the graduation "smooth-rough". It was always possible to see with the aid of a hand-lens that their surface was not so even as that of the typical bovine colonies.

The majority of the colonies belonged then to either of the following types. Some were circular or irregularly outlined, flat, small colonies with diameters ranging from 0.1 to 0.5 mm (Photo 3, to the right). Others had a much greater diameter and presented a central, somewhat raised zone, a "nucleus" and a marginal one, flat, much larger than the first, now veil-like merging itself gradually into the medium, now still quite distinct from this (Photo 6).

It was sometimes possible to observe gradual transition from the colonies of the latter description to typical rough colonies as the margin grew smaller and smaller and the "nucleus" raised higher and higher.

On the other hand when a great number of such colonies had a small "nucleus" and wide borders, these could run together so as to form a continuous layer whereon one could still see raised points which indicated the central areas of the previous single colonies.

This variety of colony-forms could be better observed on selected subcultures obtained by inoculating dilutions of suspensions prepared with layers of growth or with single colonies. It has thus been possible to select true scales from smooth to rough colonies (Photos 8 and 9).

On other occasions only with a hand-lens (magnific. x5, x10) it was possible to perceive a graduation in the roughness of the colonies between the two extremes (Photos 10 and 11).

The growth displayed by these dysgonic strains on glycerin Petragnani in a great number of transplantations from media either with or without glycerin was commonly a layer that was seldom perfectly smooth, appeared generally not so even and presented still on its surface the remnants of the previous single colonies, even after prolonged incubation. Some primary cultures isolated from the six patients referred to above showed exclusively a few isolated rough colonies, whereas another or other primary cultures from the same person displayed numerous colonies of which the great majority were dysgonic, but a few looked eugonic and were five, six times as great. Here again it was possible to perceive a gradual transition from small, slightly rough dysgonic colonies to large rough eugonic ones.

Only once a strain typed as dysgonic human and not yet accustomed to glycerin, grew on glycerin potato, giving one colony on the surface of the potato and a thick but small veil on the bouillon. Rough, luxuriously growing cultures quite undistinguishable from those of the human eugonic type were obtained by transplanting from the glycerin-potato-tube.

This growth-test on glycerin potato was repeated very often due to its importance (9) for type determination. No growth appeared as a rule for the strains isolated from three of the six patients already referred to, even after prolonged training on glycerin media. Only once for each of these three strains, the transplantation gave a growth after being repeated a great many times: for one of them eugonic growth after a year and a half subculturing on glycerin Petragnani and, for the two others, eugonic growth after two transplantations.

By subculturing from glycerin Petragnani a strain isolated from another of the six cases herein studied, an eugonic growth was obtained on glycerin potato and then maintained with the same appearance through a series of transplantations, either on potato or on Petragnani. Another strain from the same patient grew likewise dysgonically on glycerin potato on being transplanted from a primary culture on glycerin Petragnani. The repetition of the test by employing several primary cultures on glycerin Petragnani from the same cases was however always negative.

A dysgonic growth appeared on several glycerin potato tubes sown with material from glycerin Petragnani primary cultures isolated on two different occasions from the fifth case. There developed only two or three colonies that on being transplanted gave eugonic growth carried with the same aspect through a series of subcultures.

A strain isolated on glycerin Petragnani from the last case was transplanted to glycerin Bordet-Gengou and grew here into a smooth, thick layer, that on being subcultured to glycerin potato and Petragnani, gave a typical rough eugonic growth maintained then through a series of transplantations.

Another strain isolated on glycerin Petragnani grew into a thick but smooth layer and was then transplanted to glycerin Loewenstein. Here it had during three months a dysgonic appearance, forming a layer above which some small colonies rose that were sometimes mere points and it then changed its type of growth. It developed itself suddenly into a luxuriously growing culture, displaying numerous very rough colonies which rose high above the level of the medium, that is to say into an eugonic human type of culture.

The Loewenstein culture was inoculated into a guinea-pig when it still grew dysgonically; it was later recovered and went on growing dysgonically on glycerin Petragnani (Photo 7), but in such a way as to be possible to distinguish between it and a bovine culture.

The results of the animal-inoculation tests with the dysgonic strains confirmed the typing based on the study of the cultural characteristics. Twenty-six rabbits were inoculated intravenously and twelve intracutaneously. From the former sixteen were injected with 0.01 mg, two with 0.1 and eight with 1 mg of bacilli; from the latter five received 10 mg, three were inoculated with the entire growth of a culture, twenty-four days old in two cases and three months in the third, and four with sputum previously treated first with NaOH and then neutralized with H_2SO_4 . Alcohol-acid fast bacilli were found on slide preparations in all these four specimens of sputum.

Autopsy was generally carried out three months after the inoculation; a small number of animals were killed after two, four or five months.

Generalized tuberculous lesions were found only in some of the rabbits inoculated with 1 mg of bacilli isolated from two of the six cases under study. Other rabbits which were injected with strains isolated either from the same two cases or from the remainder, and in the same dose, had only isolated tubercles in the lungs. All the animals that were inoculated intravenously with the smaller doses had at autopsy either nothing or only isolated and non-caseating lesions.

The rabbits which were injected subcutaneously had at autopsy either nothing or only a localized abscess at the point of inoculation, already healed through fibrosis or then containing some thick creamy pus that on slide preparations showed only rare stabs or even only remnants of an alcohol-acid material.

COMMENT

This paper adds to the number of those which have reported the absence of bovine strains amongst tubercle bacillus cultures isolated from patients suffering from pulmonary phthisis.

As far as the human dysgonic strains are concerned the frequency in the cases herein reported does not differ very much from that quoted by Griffith

(8): "about 3 per cent of the total strains of human type". But later on Griffith (12) found no less than 28 human dysgonic strains among a group of 50 isolated in Nigeria. These findings indicate that the concept of typical and atypical strains when based only on their relative frequency may vary in different countries.

Such Nigerian strains are according to description (13) much more like those studied by us than those detailed by the "Royal Commission" appointed in England to inquire into the relations of human and animal tuberculosis. Whereas the latter "resemble bovine strains in nothing except in growing with difficulty on glycerin-agar, potato and broth" and "on serum and egg media they grow like eugonic human bacilli" (14) the former were dysgonic on all media.

Besides it was possible to distinguish between the different dysgonic strains isolated by us as far as the vigor of their growth is concerned. Thus it appears as Eastwood maintained (15) that all transitional forms are found between the bovine dysgonic and the human eugonic strains. Such scale seems to exist not only when one considers the growth as a whole but also when isolated colonies are observed.

In fact, on the one hand the dysgonic strains herein described displayed on the first transplantations and sometimes on the initial culture the most different colonies as far as the vigor of their growth and their more or less rough appearance are concerned. On the other hand the typical bovine and human strains present on the first cultures, the former perfectly smooth and dysgonic and the latter markedly rough and eugonic colonies, all forms that prevail in each case in a striking way or exist alone (for it seems that no reference is found in the literature regarding any smooth and dysgonic colony in the midst of a human eugonic culture).

The dysgonic human type that recently as we saw was isolated in a high percentage (56%) in Nigeria gained a new interest through Griffith's remark that it is more frequently found in cases of pulmonary tuberculosis in the rural districts of a certain region in Scotland than in other parts of Great Britain in a similar way to what is observed with the bovine type.

Whether or not these dysgonic strains have a peculiar rôle in the epidemiology of tuberculosis is a feature that deserves further inquiry. The number of the cases we have studied is as yet far too small to allow of any serviceable observation concerning this question.

SUMMARY

1. The type of tubercle bacillus isolated, in some cases repeatedly, from 132 patients suffering from pulmonary tuberculosis under treatment in the "Hospital S. Luiz Gonzaga", São Paulo, was determined.

2. No bovine strain was found.
3. The strains isolated repeatedly from 6 patients were classified as human dysgonic.
4. The strains isolated from the remaining patients (126) were typed as human eugonic.
5. The general characteristics of the isolated human dysgonic strains have been described, and special attention was dedicated to the morphological study of isolated colonies.

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FOTOGRAFIAS

Esclarecimentos.

- Foto 1 — Culturas iniciais de mesma idade em Petragani glicerinado.
A esquerda: raça humana eugônica.
A direita: raça C humana disgônica, mostrando crescimento em camada.
- Foto 2 — *Em baixo:* raças 157, C. e 157 disgônicas humanas.
Em cima: crescimento eugônico e rugoso da raça 71 inicialmente classificada como disgônica humana.
- Foto 3 — *A esquerda:* raça F humana disgônica com numerosas colônias, fundidas em camada lisa, na maior parte do meio.
A direita: mesma raça mostrando colônias isoladas.
- Foto 4 — Raça humana disgônica F.
 Mistura de colônias disgônicas e de colônias eugônicas e rugosas.
- Foto 5 — Raça humana disgônica 157.
 Mistura de colônias eugônicas e disgônicas; no canto inferior esquerdo e no centro colônias semelhantes às do tipo bovino.
- Foto 6 — Repiques duma mesma cultura humana disgônica (raça F).
A direita: semeadura com suspensão diluída de colônias em soro fisiológico, vendo-se colônias isoladas com "núcleo" central e película marginal.
A esquerda: semeadura com alça "carregada", vendo-se crescimento em camada.
- Foto 7 — Raça 71 classificada inicialmente como humana disgônica. Culturas provenientes dum mesmo crescimento em meio de Loewenstein.
A esquerda: cultura descendente de repique obtido do crescimento em Loewenstein quando este ainda apresentava aspecto disgônico. Crescimento disgônico pontilhado de numerosas colônias, confluídas em algumas partes do meio.
A direita: cultura isolada de cobaia inoculada com o crescimento em Loewenstein após sua transformação em cultura eugônica. Crescimento eugônico e rugoso.
- Fotos 8 e 9 — Raças 157 e 71 humanas disgônicas.
 Transição "liso-rugoso" de colônia a colônia duma mesma cultura.
- Fotos 10 e 11 — Raças 57 e 157 humanas disgônicas.
 Transição de colônia lisa para rugosa.

Explanations of the photographs

- PHOTO 1 — Primary cultures of the same age on glycerin Petragani.
To the left: human eugonic strain.
To the right: human dysgonic strain C, showing a layer of growth.
- PHOTO 2 — *Below:* human dysgonic strains 157, C and 157.
Above: eugonic and rough growth of the strain 71 at first classified as human dysgonic.
- PHOTO 3 — *To the left:* human dysgonic strain F showing numerous colonies that ran together in the greatest part of the medium.
To the right: the same strain showing isolated colonies.

- PHOTO 4 — Human dysgonic strain F.
Mixture of dysgonic colonies and of eugonic and rough ones.
- PHOTO 5 — Human dysgonic strain 157.
Mixture of eugonic and dysgonic colonies. On the lower left corner and in the middle some colonies resembling those of the bovine type.
- PHOTO 6 — Transplants of the same human dysgonic culture (strain F).
To the right: seeding carried out with a diluted suspension of colonies in saline; one sees isolated colonies with central "nucleus" and marginal veil.
To the left: seeding carried out with a loopful of bacilli; one sees a layer of growth.
- PHOTO 7 — Strain 71, at first classified as human dysgonic. Both cultures came from the same growth on Loewenstein medium.
To the left: transplant from a culture that came from the Loewenstein culture when this grew still dysgonically. Dysgonic growth dotted with numerous colonies that ran together in some parts of the medium.
To the right: culture recovered from a guinea-pig that was inoculated with the Loewenstein growth after this had changed into an eugonic culture. Eugonic and rough growth.
- PHOTOS 8 AND 9 — Human dysgonic strains 157 and 71.
"Smooth-rough" transition from colony to colony of the same culture.
- PHOTOS 10 AND 11 — Human dysgonic strains 57 and 157.
Transition from smooth to rough colony.



Foto 1

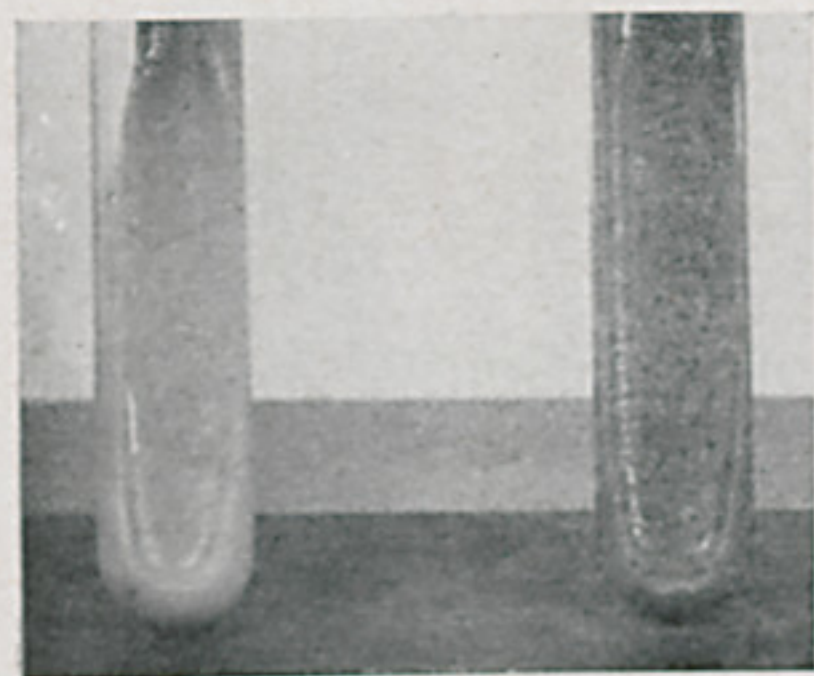


Foto 3

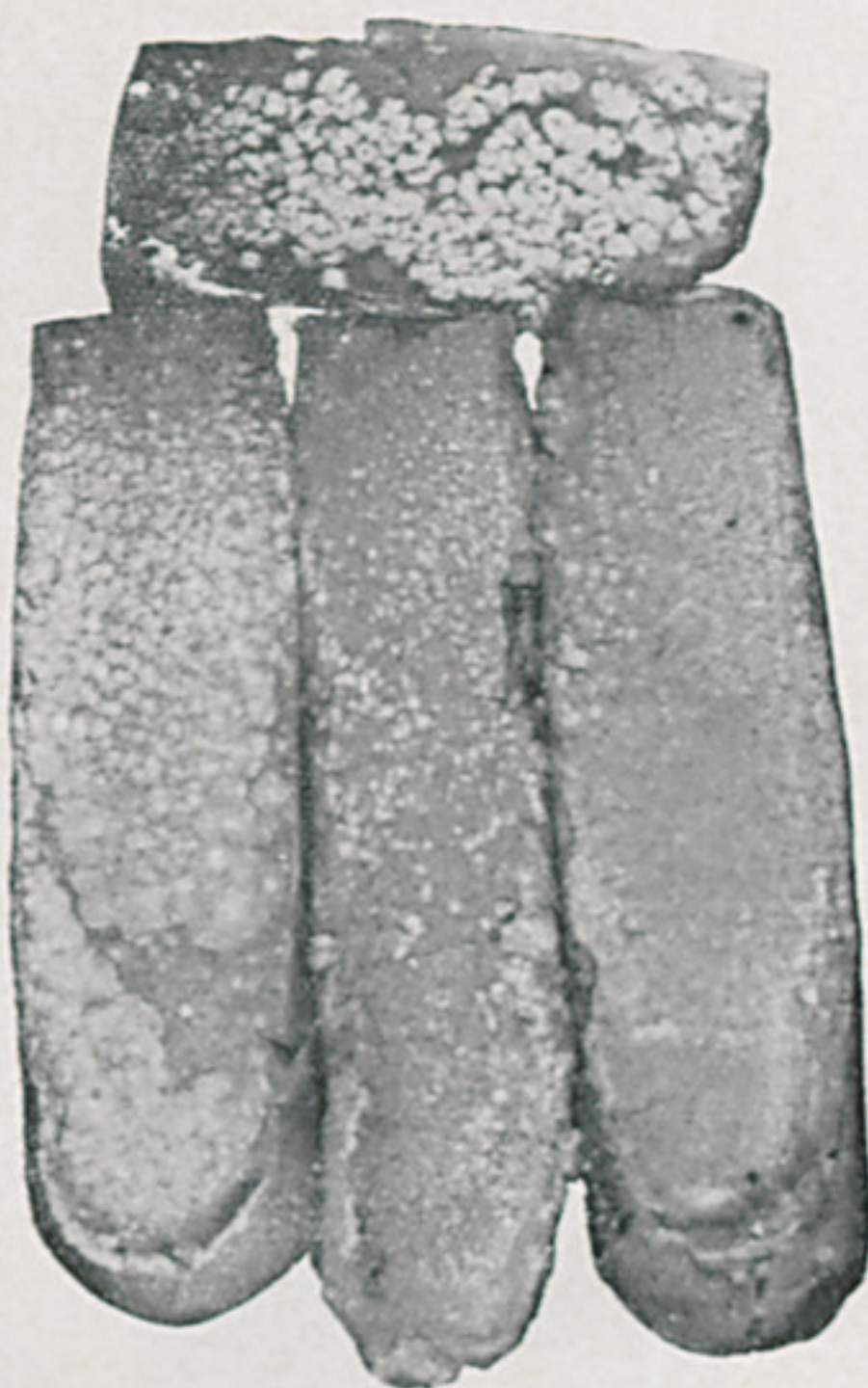


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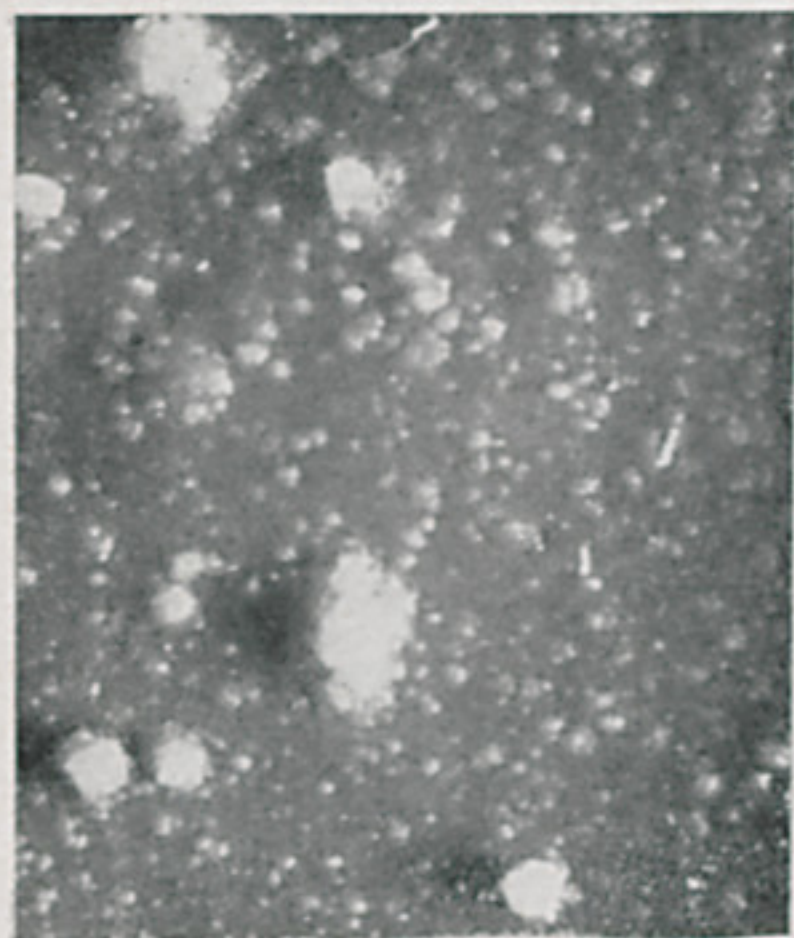


Foto 4



Foto 5

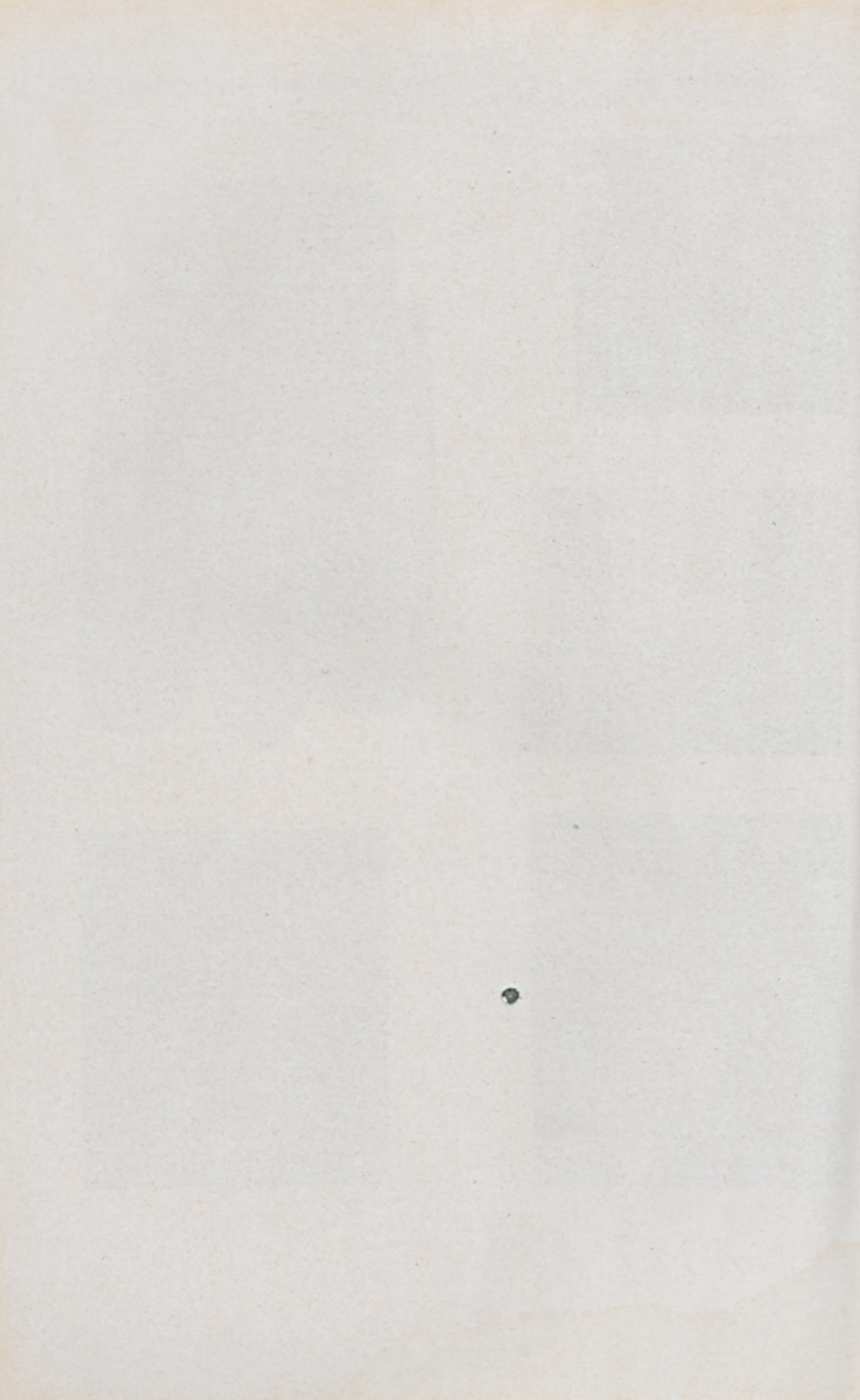




Foto 6

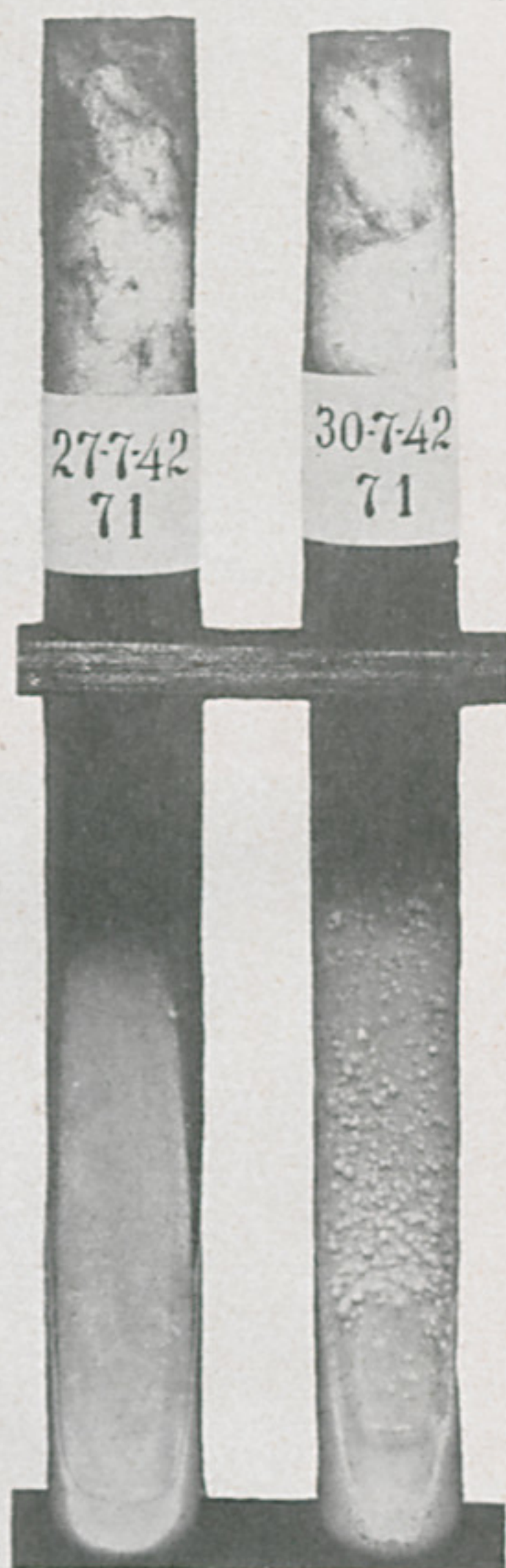


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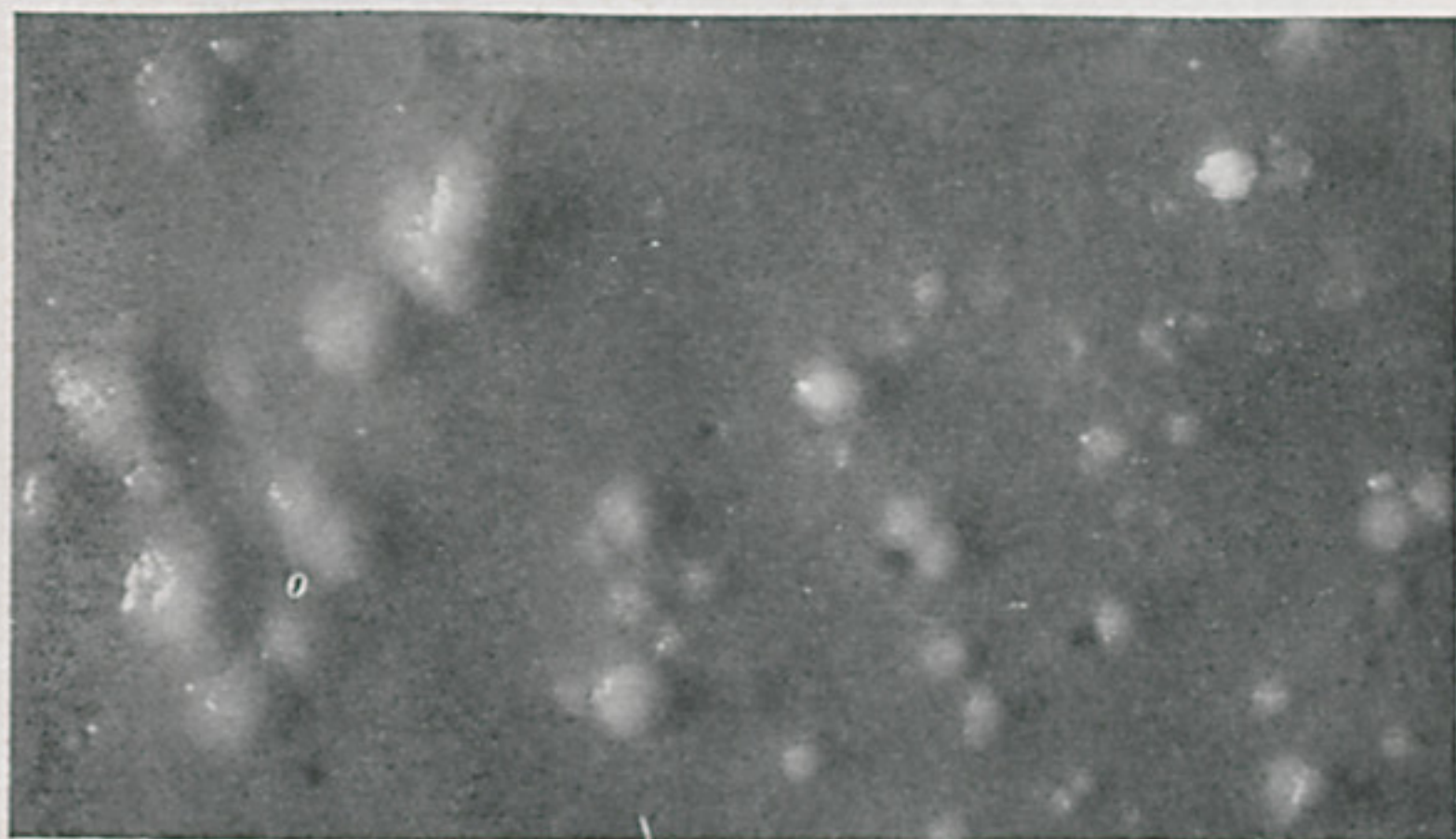


Foto 8

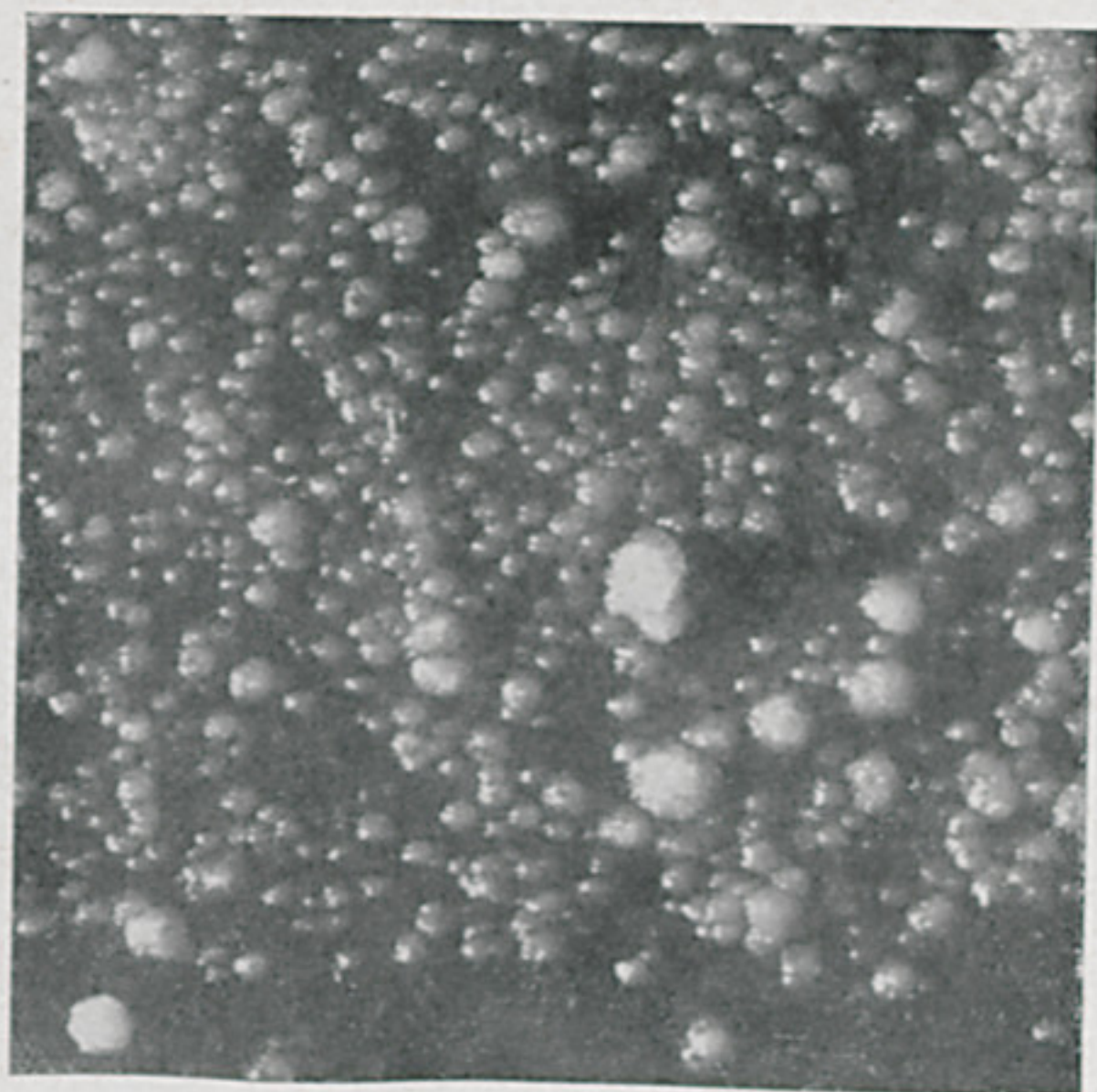


Foto 9

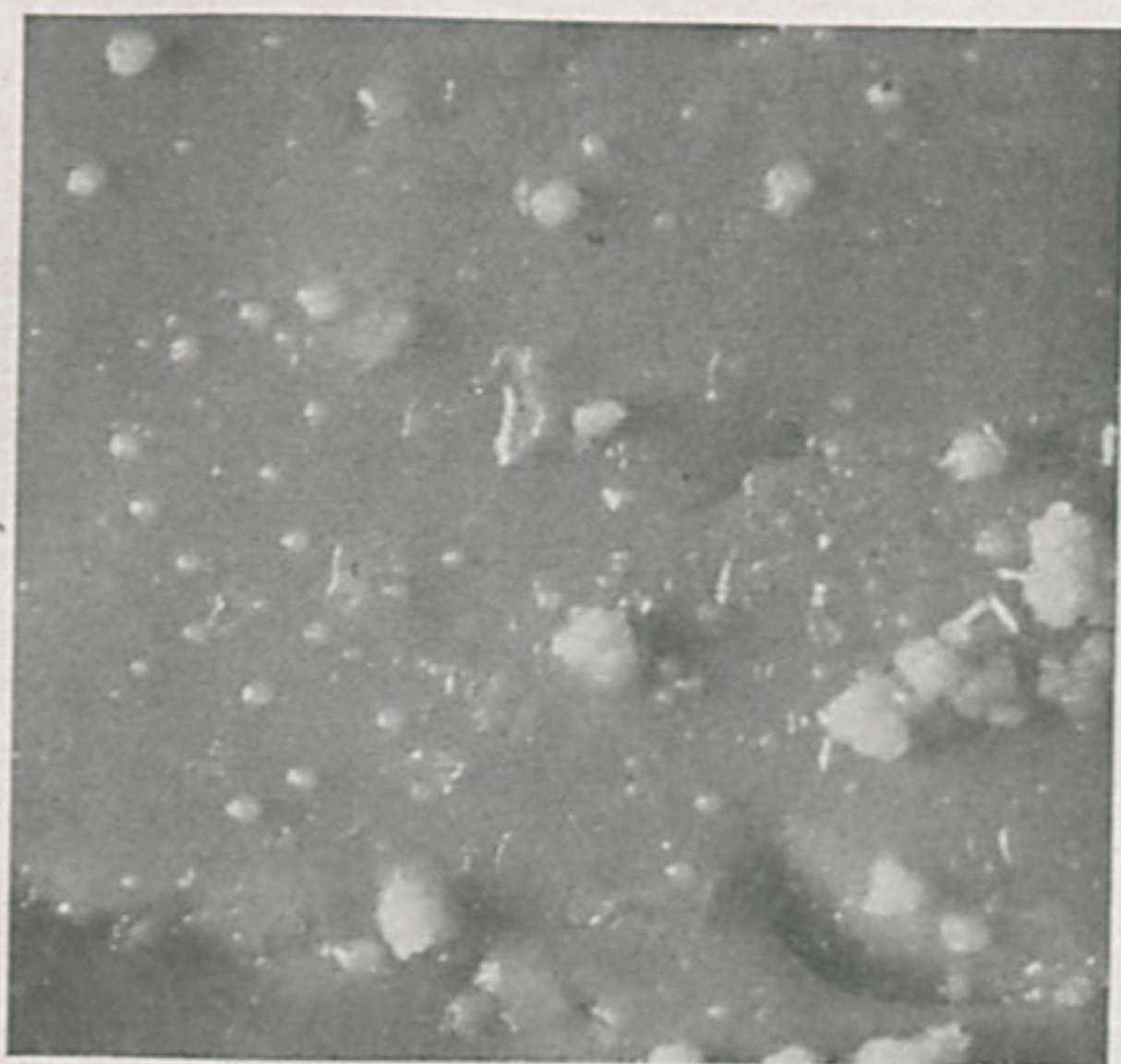


Foto 10

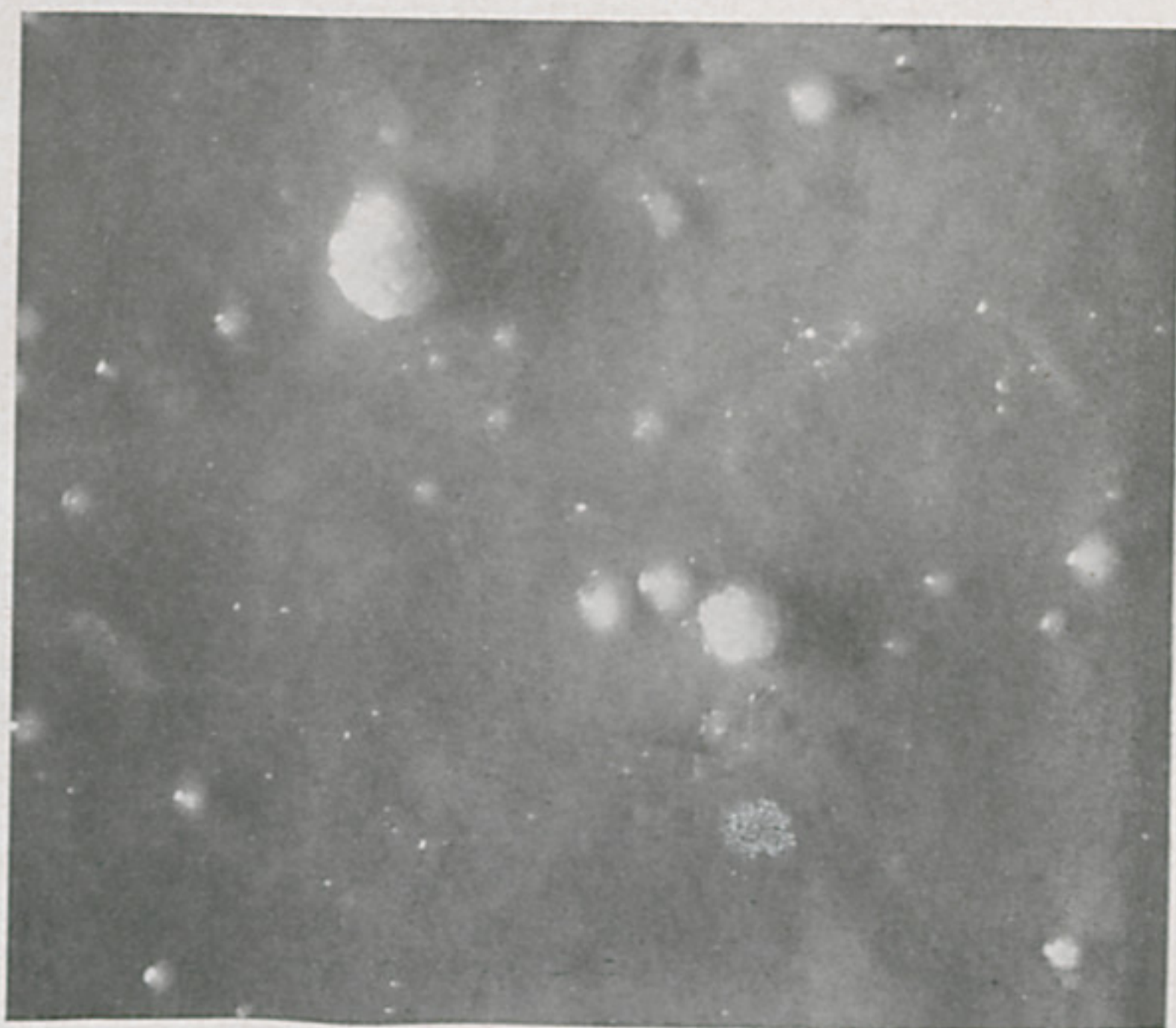


Foto 11

CONTRIBUIÇÃO À MATÉRIA MÉDICA VEGETAL DO BRASIL

8. Complemento ao estudo farmacognóstico e terapêutico das várias espécies do gênero *Jacaranda* (*Bignoniaceae*)

POR

FERNANDO PAES DE BARROS

I

Entre as famílias botânicas mais ricas em espécies medicinais está, incontestavelmente, a das *Bignoniaceae*, na qual assume preponderância, pelo número e pelas múltiplas propriedades terapêuticas dos vegetais que nele se grupam, o gênero *Jacaranda*, cujo estudo foi iniciado pelo incansável pesquisador que era Theodoro Peckolt e continuado pelo seu neto, Waldemar Peckolt, no Instituto Butantan.

O conhecimento empírico das virtudes curativas das jacarandas vem de era remota. Na América do Sul, e principalmente no Brasil, os colonizadores europeus vieram encontrá-lo já divulgado entre as nações indígenas, mas foi muito mais tarde, em 1866, que êsse conhecimento se difundiu, cientificamente, pelos trabalhos do velho Peckolt, que foi quem iniciou no Brasil as análises químicas das plantas tidas como de utilidade médica.

Tão ricas de propriedades medicinais são as plantas que se classificam no gênero *Jacaranda*, que julgamos dever prosseguir no seu estudo, do ponto de vista terapêutico. O presente marca o início desse tentamen e tem como objetivo uma homenagem mais a Waldemar Peckolt.

Conhecendo, demonstrado pelo seu avô, o grande valor das jacarandas, êste nosso antecessor e organizador da Seção de Botânica Médica do Instituto Butantan, passou a se dedicar ao estudo das mesmas, e, especialmente, à espécie por êle tida como sendo a *Jacaranda decurrens* CHAM., porém, guiou as suas experiências para um lado ainda não explorado anteriormente, o das proprieda-

des parasitcidas da planta. Prosseguindo com êste objetivo, W. Peckolt o attingiu plenamente, colhendo os resultados positivos que publicou nas "Memórias do Instituto Butantan" com o Dr. Alcides Prado, do serviço de parasitologia, que se encarregou da parte referente a essa especialidade com toda a proficiência.

Entretanto, um engano, que de forma alguma poderá desmerecer o trabalho daquele cientista, foi por êle cometido, trazendo isso alguma confusão, por ser um equívoco de ordem sistemática, que precisa ser reparado.

Embora continuem desaparecidos os cadernos de notas deixados pelo saudoso pesquisador, por vários dados por nós encontrados, ao tomarmos conta da sua Seção, podemos dizer que a espécie que serviu para os estudos e experiências de W. Peckolt, nos seus trabalhos químicos e farmacêuticos, de que resultou a descoberta de um novo tratamento, seguro, fácil e econômico, das amibioses e de muitas outras protozooses intestinais, não foi a *Jacaranda decurrens* CHAM., conforme êle julgou, mas sim a *Jacaranda caroba* (VELL.) P. DC.

A simples observação das figuras esclarece a questão.

E' em homenagem à memória e ao saber daquele nosso ilustrado colega que fazemos esta correção, necessária para completar o seu valioso trabalho. Ninguém, por um simples equívoco na classificação de um vegetal, cousa bastante comum, mesmo entre os que se dedicam somente à botânica, poderá por em dúvida a capacidade científica de um pesquisador, cuja atividade se repartia entre a química, a farmacologia e a clínica médica.

Waldemar Peckolt era tudo isso e, por essa razão, a humanidade muito lhe deve; basta, si mais não houvesse feito, esta sua descoberta que tem restituído a saúde a centenas de doentes condenados a tratamentos penosos e grandemente dispendiosos. Foi um dos últimos trabalhos que realizou, quando o seu organismo, já depauperado, lutava contra o mal que, não muito longe, iria por termo a sua atividade terrena.

E' também ainda em homenagem àquele valoroso colega que aproveitamos a oportunidade de entrarmos na sua tão apreciada seara para fazer a retificação de um lapso verificado na publicação do mesmo trabalho de Peckolt, que escapou à revisão, do qual, temos quasi certeza, não cabe culpa ao autor.

Na sua publicação — VI. *Estudo pharmacognostico e therapeutico da Jacaranda decurrens Cham. (Bignoniaceae)* MEMÓRIAS DO INSTITUTO BUTANTAN, tomo IX, 1935, à página 307, primeira linha — está escrito: "A carobina é uma glicosida, que não nos foi possível obter crystallizada, sinão amorpha."

Mais abaixo, na mesma página, encontra-se o seguinte: "Filtra-se e evapora-se o residuo até crystalisar, em temperatura que não exceda a mais de 80° C. Separam-se os crystaes do liquido...".