Conclusion:

Clinical observations suggest the factor of auto-intoxication in catatonia. Therefore a series of experiments have been begun in our laboratory concerning the possibility of the production of catatonia through auto-intoxication in experimental animals.

Some of these experiments with ligatures of the intestine produced pronounced positive results.

We may remember, that in human pathology the hypothesis of enterogenesis in dementia praecox, has been proposed (f.e. by BUSCAINO) [1].

Since experimental catatonia can be produced in many ways, it may not be concluded from the above, that human catatonia results, in every instance from intestinal-intoxication. Therefore, the other biological possibilities of experimental catatonia must be investigated. Such experiments are now in the process of investigation.

[1]) V. M. BUSCAINO: "Les récherches récentes sur l'étiologie et la pathogénie de la confusion mentale et de la démence précoce". Encéphale, janvier 1930, p. 48.

Medicine. — *On the seasonal longevity of Anopheles maculipennis in Holland with reference to their ability to act as malarial vectors.* By A. DE BUCK and N. H. SWELLENGREBEL. (From SWELLENGREBEL's Laboratory in the Institute of Tropical Hygiene [Director Prof. Dr. W. A. P. SCHÜFFNER] of the Royal Colonial Institute at Amsterdam). (Communicated by Prof. W. A. P. SCHÜFFNER).

(Communicated at the meeting of February 23, 1935.)

1. *Introduction.*

JAMES, NICOL and SHUTE [1]) have shown that the rate of survival in batches of infected anopheles (i.e. the percentage still alive on the day sporozoites appear in the salivary glands) varies with the month in which they have been infected. The rate of survival is high (about 50 %) from August till November, dropping to about 30 % in December and January, still lower (about 20 %) in February and March, to attain the lowest level (less than 10 %) in April and May. In June and July it starts rising again but not above the level reached in February and March. According to these data the year can be divided into two seasons, one is favourable to malariatransmission: August to November, the other is not: December

[1]) JAMES, NICOL and SHUTE: Medicina de los Países Cálidos, I, 1928, pp. 161—164; Transact. Far East. Ass. Trop. Med. 7th Congress, Vol. II, 1929, pp. 712—717.

JAMES: Transact. Roy. Soc. Trop. Med. and Hyg. XXIV, 1931, No. 5, p. 491; Proc. Roy. Soc. Med. XXII, 1929, Sect. Epid. and State Med., pp. 71—85.

to July, the second one including the greater portion of the period of sexual activity of the mosquitoes. This sexual activity, endangering life by the ovulation and parturition it entails, seriously impedes malarial transmission by mosquitoes in the laboratory and still more so in nature, where anopheles are threatened by the dangers consequent upon the long flights they are forced to take for the sake of oviposition. Not until the moment sexual activity is over and they find an undisturbed shelter in human dwellings, anopheles attain that particular position JAMES had postulated as the one indispensable for a truly effective malarial transmission.

In Holland JAMES' results were particularly interesting, not only because they agree with observations collected there but also because they have opened vistas of an entirely new interpretation of these observations.

We knew already as a broad rule that anopheles (*A. maculipennis* var. *atroparvus*) in Holland are mostly found malaria-infected in September—December, a period practically coinciding with JAMES' first (favourable) season. Infected mosquitoes are rare during the time the malarial season reaches its acme, viz. in May—July, i.e. a time included within JAMES' second (unfavourable) season. In agreement with ROUBAUD's [2]) views, we interpreted these observations by assuming that infected anopheles are just as numerous in summer as in autumn but that they cannot be found in summer in human dwellings because: 1. anopheles do not stay there as they have always to leave them for the sake of oviposition; 2. infected anopheles are speedily lost in the inhabited stables which attract them much more than human dwellings, on their return from the breedingplaces. This "stabular attraction" is no longer available in autumn as it influences none but anopheles on their returnflight from the breedingplaces. In autumn there are no such flights as oviposition has stopped, although blood-feeding continues. Anopheles stay where they happen to be when the process of ovulation comes to a close: in the stables where they are numerous but harmless; in human dwellings where they are comparatively rare but where one single specimen is of more value to malariatransmission than a hundred in summer.

JAMES' observations admitted of a different interpretation of the observations made in Holland: It is not their being lost in stables, which renders it difficult to find infected anopheles in summer, but their being actually rare because of the increased mortality at that time of the year.

2. *Longevity of infected anopheles caught in nature and infected in the laboratory.*

Table No. 1 and the black columns of the diagrams No. 1 and 2 are showing month by month: *a.* the number of anopheles infected by causing them to bite a carrier of benign tertian gametes on a certain day of that month; *b.* the number surviving on the day (usually the 10th—12th after the infecting meal) the first specimen of each separate batch of mosquitoes was found harbouring salivary sporozoites; *c.* the number surviving 4 weeks after the infecting meal. We have added this third column for

[2]) ROUBAUD: C. R. Ac. d. Sciences, Paris, CLXXXVI, No. 5, pp. 329—331.

TABLE I. Anopheles maculipennis var. atroparvus *caught in nature* experimentally infected with benign tertian malaria.
Rate of survival.

Month	Rate of survival at the time salivary infection had become established			Rate of survival four weeks after infecting meal		
	Total of anopheles biting gamete carrier [1]	Anopheles still alive on the day salivary infection was first detected		Total of anopheles biting gamete carrier [2]	Anopheles still alive four weeks after infecting meal	
		Total	Percent (rate of survival)		Total	Percent (rate of survival)
Jan.	420	265	63 %	241	83	34 %
Febr.	433	287	66 %	367	130	35 %
Mrch.	496	291	59 %	411	116	28 %
Apr.	849	374	44 %	817	106	13 %
May	190	45	24 %	189	16	8 %
June	207	106	51 %	205	40	20 %
July	328	190	58 %	230	30	13 %
Aug.	607	389	64 %	455	88	19 %
Sept.	251	202	80 %	172	108	62 %
Oct.	354	276	78 %	243	101	41 %
Nov.	635	481	76 %	470	302	64 %
Dec.	352	270	77 %	406	225	55 %

[1]) Not including mosquitoes sacrificed to ascertain the presence of salivary infection.
[2]) This total is less than that in the first column because a number of mosquitoes had been sacrificed for various reasons after salivary infection had become established.

practical considerations, arguing that sporozoites in Holland take a longer time in maturing under natural conditions, than they do in our "tropical chamber" at 80° F. and, moreover, that it is no use for anopheles to have their salivary glands invaded by sporozoites unless some additional time is allowed for their transmitting the infection.

The anopheles referred to in Table I, II and Diagram No. 1 and 2, were *A. maculipennis* var. *atroparvus*. They had been allowed to bite gamete-carriers once, rarely twice or more times, after which they were transferred to the "tropical chamber" (temperature 80°, relative humidity 90 %), where each batch of mosquitoes was kept separate in cages of 2,4 cub. ft. and fed on sugar-water. By the time sporozoites might be expected to make their appearance in the salivary glands, a few mosquitoes were sacrificed out of each batch. When salivary infection was detected on a certain day (usually the 10th—12th), the rate of survival was computed for that particular day, after which the cages were transferred to an unheated room (temperature 60°—75°

in summer, 50°—52° in winter, 37°—39° during frosts). Feeding on sugar-water was continued unless the mosquitoes were put into requisition to infect a patient. The rate of survival was established a second time 4 weeks after the infecting meal, i.e. 16—18 days after salivary infection had first been detected and anopheles had been transferred from tropical climate to one natural to Holland.

Not all anopheles surviving on the 10th—12th day were found infected. Their sporozoite-rate was 72 % as established by the dissection of 2042 specimens. Within the period of March—October this rate was 68 %.

On the whole these results well agree with JAMES'. It is true our rates of survival are all higher than his, the minimum is not so low and the rate of survival in Sept.—Oct. is not so much above that during the beginning of the year. As a consequence the favourable and unfavourable seasons are less distinctly separated. This, however, is true only of the rates of survival computed at the time salivary infections make their appearance. The rates established on the 28th day after the infecting meal (and they are the ones which are of really practical importance) are in complete agreement with JAMES' figures: a favourable season from September till December (rate of survival 59 %), an unfavourable one from April till August (rate of survival 15 %) joined by a period of transition in January—March (rate of survival 32 %). The sporozoite-rates of anopheles infected within these three periods are about the same, viz. 74 %, 70 % and 71 % respectively. So the summer mosquitoes are indifferent malaria vectors not because they are bad carriers but because they are too short-lived.

3. *Longevity of infected anopheles bred in the laboratory.*

Keeping alive anopheles caught in nature (in stables around Amsterdam) was found to be most difficult during the final stages of the life of the hibernating mosquitoes, when they leave their wintershelters to deposit their ova and, then, to die. In May, for instance, the rate of survival was 24 % only on the day salivary sporozoites appeared and as low as 8 % on the 28th day. So we resolved to try whether laboratory-bred anopheles would do better and they answered our purpose so well that we continued their use during the whole of the unfavourable period.

In March—April and in September—October we bred our anopheles in the tropical chamber, in May—August in an unheated room. The sporozoite-rate, established in 477 specimens, was found to be 63 % which is about the same as in anopheles caught in nature and infected during the same months.

Table II and diagrams 1 and 2 (white columns) show rates of survival on the first appearance of salivary infection two to three times higher in laboratory-bred mosquitoes infected in April and May than in the hibernating generation caught in stables and infected during the same period. In June and July the superiority of laboratory-bred anopheles

TABLE II. Anopheles maculipennis var. atroparvus *bred in the laboratory* experimentally infected with benign tertian malaria.

Rate of survival.

Month	Rate of survival at the time salivary infection had become established.			Rate of survival four weeks after infecting meal		
	Total of anopheles biting gamete carrier [1])	Anopheles still alive on the day salivary infection was first detected		Total of anopheles biting gamete carrier [2])	Anopheles still alive four weeks after infecting meal	
		Total	Percent (rate of survival)		Total	Percent (rate of survival)
Mrch.	20	17	85 %	19	16	84 %
Apr.	78	77	99 %	64	42	66 %
May	233	154	66 %	159	47	30 %
June	219	165	75 %	171	65	38 %
July	162	139	86 %	125	93	74 %
Aug.	62	44	71 %	72	35	49 %
Sept.	352	335	95 %	340	265	78 %
Oct.	61	52	85 %	64	29	45 %

[1]) See note [1]) Table I.
[2]) See note [2]) Table I.

over wild ones becomes less marked, and in August—October there is little difference between the two groups.

The superiority of laboratory-bred anopheles is even more marked and extends over a longer period when measured by the rate of survival after 28 days: in April—May and in July—August it is three to five times greater than in wild anopheles and it is not till September—October that the two groups become almost equal in this respect.

Here it is no longer the revived hibernating wild generation only which is inferior to the laboratory-bred anopheles infected simultaneously, but likewise the wild summergeneration as far down as August, i.e. anopheles weakened by the wear and tear of an outdoor life during the period of sexual activity, to which they had been exposed previous to their being infected with malaria and from which the laboratory-bred generation had been protected.

It may be noticed that the laboratory-bred anopheles show a drop in their rate of survival over 3 successive months (April—June), As the numbers of laboratory-bred anopheles we worked with are necessarily much smaller than those of the wild anopheles, we are not sure whether this drop in spring and early summer has any more significance than the ones observed in August and October. If it has, it evidently cannot be

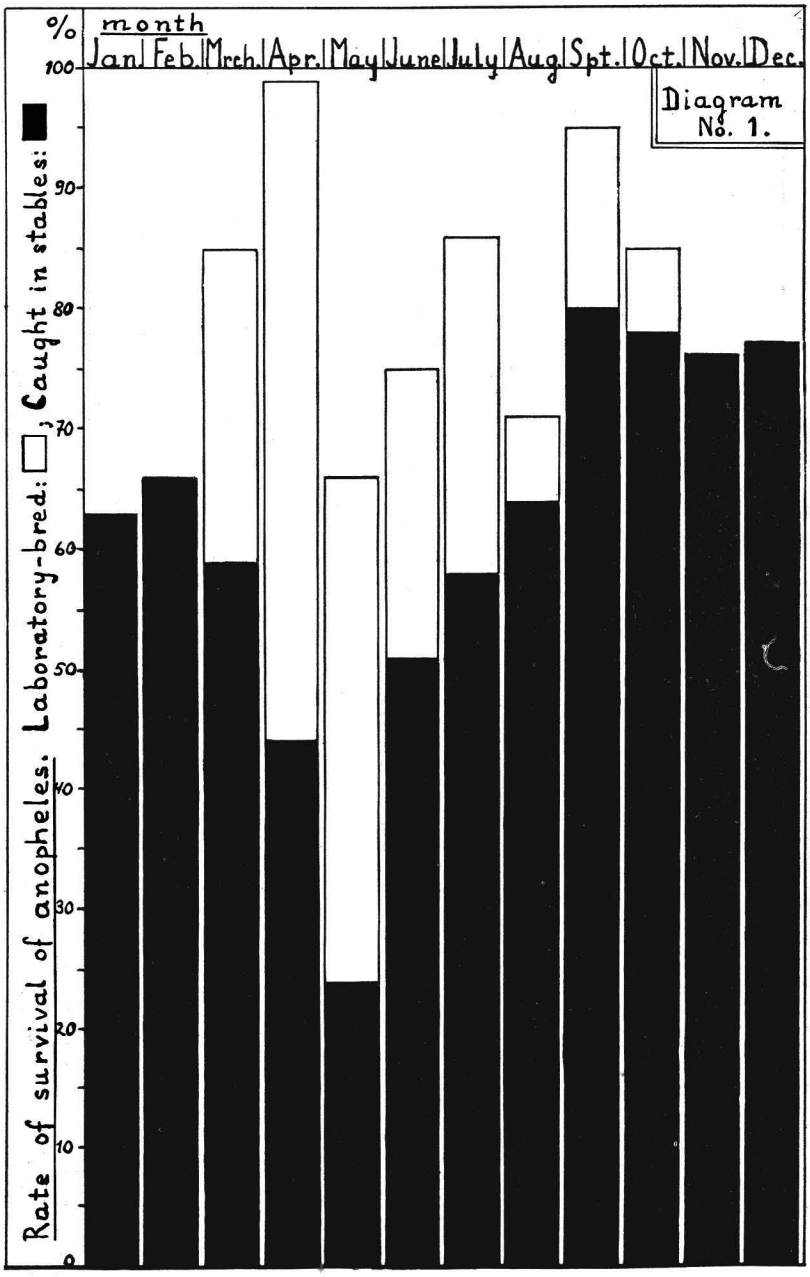

Diagram No. 1.

Monthly rate of survival at the moment sporozoites make their appearance in the salivary glands of:

a. Anopheles caught in stables (black columns).

b. Laboratory-bred Anopheles (white columns).

Note: The black columns stand in front of the white ones, hiding the basal portion of the latter.

explained — as it was in wild anopheles in April and May — by the debility of the rearguard of the hibernating generation. For the laboratory-bred anopheles in those months are equal to the first summergeneration. The change in breeding conditions occurring in May (from tropical chamber to unheated room) may be partly responsible for this lessened rate of survival of laboratory-bred anopheles.

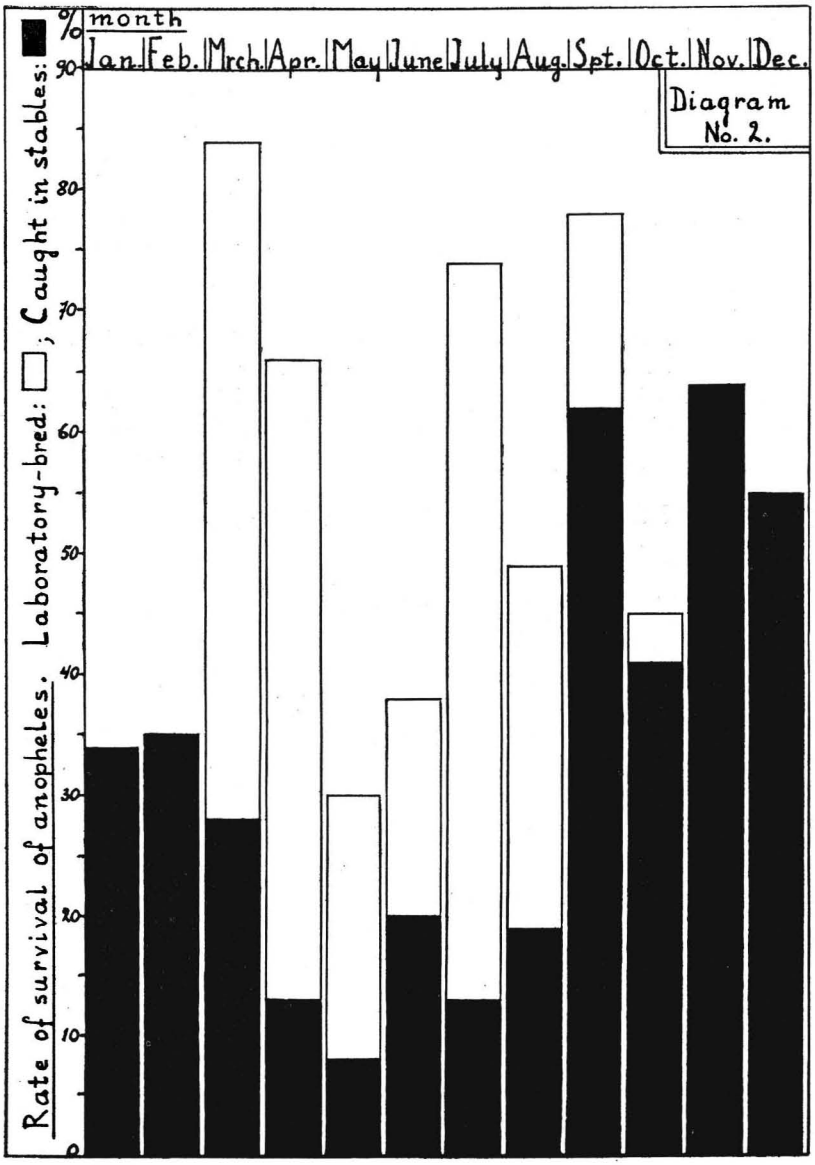

Diagram No. 2.

Monthly rate of survival four weeks after the infecting meal of:

a. Anopheles caught in stables (black columns).

b. Laboratory-bred Anopheles (white columns).

Note: The black columns stand in front of the white ones, hiding the basal portion of the latter.

4. *Longevity of heavily and slightly infected anopheles.*

In the foregoing no account has been taken of the degree of infection of anopheles as estimated by the number of oocysts on the stomach. In the following account, referring to anopheles caught in nature only, we are going to mend this defect by considering separately the longevity of anopheles suffering from:

1. Heavy infections: i.e. batches with 93 % of the mosquitoes infected, 91 % of these with more than 20 oocysts, 80 % with 100 oocysts or over, average per infected mosquito: 205 oocysts.
2. Slight infections: i.e. batches with 31 % of the mosquitoes infected, 18 % of these with more than 20 oocysts, none over 80, average per infected mosquito: 12 oocysts.

As the mosquitoes were infected for practical purposes (treatment of G. P. I.) we had to be careful not to sacrifice too many of them for the sake of assessing the degree of infection. So the number of mosquitoes examined in this way is necessarily much smaller than the group dealt with in the preceding paragraphs. For that reason their seasonal arrangement had to be in groups comprising three months instead of one.

The result of this arrangement is shown in Table III and Diagram 3.

TABLE III. Anopheles maculipennis var. atroparvus caught in stables infected with benign tertian malaria.

Rate of survival.

Three-monthly period.	Rate of survival at the time salivary infection had become established. (see Diagram 3 A)			Rate of survival four weeks after infecting meal. (see Diagram 3 B)		
	Total of anopheles biting gamete carrier	Anopheles still alive on the day salivary infection was first detected		Total of anopheles biting gamete carrier	Anopheles still alive four weeks after infecting meal	
		Total	Percent (rate of survival)		Total	Percent (rate of survival)
A. Heavily infected anopheles.						
Ja.—Mr.	137	105	77 %	133	10	7 %
Ap.—Jn.	128	46	36 %	128	6	5 %
Jl.—Sp.	114	72	63 %	111	28	25 %
Oc.—Dc.	388	303	78 %	365	191	52 %
Total	767	526	68 %	737	235	32 %

TABLE III. (Continued).

B. Slightly infected anopheles.

Ja.—Mr.	896	545	61 %	682	223	33 %
Ap.—Jn.	583	193	33 %	529	48	9 %
Jl.—Sp.	457	346	76 %	284	140	49 %
Oc.—Dc.	624	435	70 %	608	302	50 %
Total	2560	1519	59 %	2103	713	34 %

Taking account of the totals only, the difference between the two groups seems to be negligible. But the quarterly figures separately show

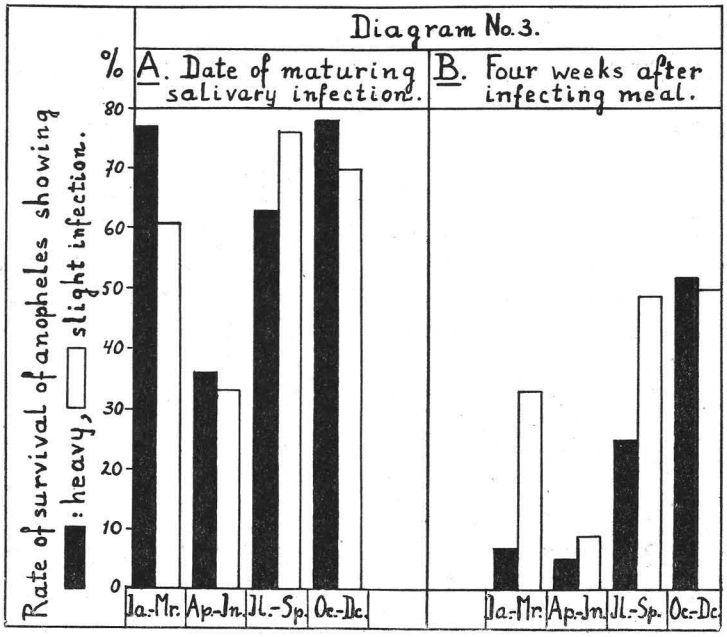

Diagram No. 3 A and 3 B.

Quarterly rate of survival in anophèles caught in stables:

Diagram 3 A. At the moment sporozoites make their appearance in the salivary glands.

Diagram 3 B. Four weeks after the infecting meal.

Black columns: heavily infected anopheles.

White columns: slightly infected anopheles.

a considerable difference in the rate of survival of the two groups four weeks after the infecting meal. The heavily infected group lags much behind in the first and third quarter, in the fourth only (Oct.—Dec.) it keeps up with the slightly infected group. This difference, however, is

not yet apparent at the time of first apparition of salivary sporozoites. if anything the heavily infected group is slightly in the advantage.

So we conclude that there exists a greater mortality in heavily infected anopheles than in slightly infected ones. Whatever the cause may be of this increased mortality it is acting only during the unfavourable season of February—August and it affects the mosquitoes suffering from a salivary infection. Intestinal infection alone, however severe, does no apparent harm.

Conclusions.

These results, confirming JAMES, NICOL and SHUTE's obtained in England, and agreeing with observations in Holland on the presence of infected anopheles in human dwellings during autumn, emphasize the epidemiological importance of the autumnal mosquitoes by showing that the scarcity of summer infections in anopheles is not to be explained by stabular attraction only, but likewise by the short span of life of the reawakened wintergeneration and the subsequent summergenerations.

The comparison of anopheles bred in the laboratory and caught in nature confirms JAMES' views on the special importance as malariavectors of anopheles protected from the dangers of an outdoor life.

The comparison of heavily and slightly infected anopheles, moreover, shows the menace to anopheline existence in spring and summer to be particularly serious for heavily infected mosquitoes.

Botany — *Rektifikation zu meiner Arbeit „Speichel-, Pankreas- und Aspergillusamylase (Taka-Diastase) als Gemisch zweier Arten von Amylasen.* Von G. GIESBERGER. (Communicated by Prof. F. A. F. C. WENT)

(Communicated at the meeting of February 23, 1935.)

In einer früheren Arbeit (Proc. Kon. Akad. v. Wetensch. Amsterdam, 37, 336) war ich auf Grund einiger Diffusionsversuche mit Speichel-, Pankreas-, und Aspergillusamylase zu dem Schlusse gekommen, dasz diese Amylasen als ein Gemisch zweier Amylasen aufzufassen sind. Das Diffusionsfeld in stärkehaltiger Gelatine zeigte nämlich nach Behandlung mit Jodjodkalium ein farbloses Zentrum, das von einem purpurnen Ringe umgeben war. Als ich aber vor kurzem diese Diffusionsversuche wiederholte, konnte ich oft nur einen hellblauen Ring bekommen; bisweilen