ON THE SUPERSENSITATION OF PERSONS SUFFERING FROM DIPHTHERIA BY REPEATED INJECTIONS OF HORSE SERUM.

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When the blood serum of one animal is injected into an animal of a different species the injected animal in many instances appears to take no hurt. If however after a certain interval the experiment is repeated, it has been noted that the injected animal may speedily show evidence of physical disturbance. Its breathing becomes rapid: its heart-beat grows feeble: its limbs move spasmodically and general convulsions may ensue. These phenomena have been taken to indicate that the injected animal has been *supersensitized*—or rendered abnormally sensitive—to the serum employed. They may attain such gravity that the animal dies. It has been suggested that the same untoward issue is possible in the case of man, and that the death of patients under treatment for diphtheria is to be apprehended in certain circumstances as the result of repeated injections of antidiphtherial serum, for the reason that the serum in question is derived not from the human subject but from the horse. A suggestion which assails the prestige of the antitoxin treatment of diphtheria cannot be viewed with indifference: it must either be sustained or rejected.

The narratives of the cases of persons who have received such repeated injections are criteria for the discussion of the question whether the mischance suggested is an imminent danger or is merely a speculative possibility. It thus seemed appropriate to extract from a continuous series of reports such information as was pertinent in the matter. It is the purpose of this paper to record the effects of repeated injections of horse serum in persons associated with the City of Glasgow Fever and Small-pox Hospitals at Belvidere.

https://doi.org/10.1017/S002217240003312X Published online by Cambridge University Press

It may be permitted by way of initial survey to recall the work of certain observers, to refer in the first place to experiments on supersensitation, or production of excessive susceptibility, in animals by bacterial products and by extraneous sera, and in the second place to investigations of the corresponding phenomena in the human subject.

Supersensitation of animals to bacterial products.

Brieger[6] (according to Otto, p. 9) relates the case of a goat which died of typical tetanus, while highly immunized against the toxin of that disease.

Von Behring and Kitashima (1901) record the death of a horse in the course of immunization against the diphtheria toxin, despite the high antitoxic content of its blood.

Rist (1903) narrates the effects produced in guinea-pigs by the repeated injection of ·01 to ·05 cg. of dried diphtheria bacilli. In these experiments the first dose caused illness and recovery ; the second dose caused illness with a slower recovery ; the third dose was followed by death.

It was demonstrated by Kretz [10] that an estimated mixture of toxin with antitoxin which was neutral when injected into normal animals induced a reaction in animals which had been actively immunized to the toxin.

The repeated tuberculinization of tuberculous cattle was stated by Nocard[12] to effect a genuine tolerance. Vallée[19] was led to an opposite view by experiments which are traversed by Arloing, but which seem to indicate that a second or third injection of tuberculin into a tuberculous ox produces a reaction which is earlier in its appearance and more rapid in its course than the reaction which follows a first injection.

It emerges from the instances related that an animal under certain circumstances may be supersensitized, or rendered more sensitive than normal, to the action of bacteria and their products, and that the condition so produced may be displayed either in a fatal issue, or in the occurrence of a phenomenon which would otherwise fail to appear, or in the earlier onset of the reaction induced.

Supersensitation of animals to extraneous sera.

Arthus (1903) conducted a series of experiments in which the repeated injection of rabbits with normal horse serum produced a specific supersensitation. One rabbit died of the intravenous injection of 2 c.c. of horse serum. Under this reference Arthus suggests the possibility of danger to man by similar treatment.

A further inquiry by Arthus with Breton (1903) demonstrated the occurrence of severe cutaneous lesions in rabbits supersensitized to horse serum, as a result of repeated injections of the serum.

Wolf (cited by Otto, p. 11) has shown that the repeated injection of extraneous serum does not produce tolerance: on the contrary the animals progressively

deteriorate. He regards the condition as supersensitation to a specific serum and not as an impairment of defence against noxious substances in general.

The researches of Battelli (1905) are concerned with blood corpuscles. They may however be noted here. These researches indicate that the extractives of dogs' or guinea-pigs' blood corpuscles which are not toxic to normal rabbits are capable of causing the death of rabbits which have been immunized against dogs' or guinea-pigs' blood corpuscles.

It appears by these records of the repeated injection into animals of the sera and blood extractives of animals of a different species that a condition of supersensitation results in the injected animals, which is similar in character to that which follows the repeated injection of bacterial products.

Reference may be made in more detail to the recent inquiry of Otto[13] which is concerned with diphtheria toxin and with horse serum. Six series of experiments on guinea-pigs constitute this inquiry. The deaths narrated occurred as a rule within an hour or thereby of the second injection.

Series I, establishing the phenomenon, which is associated by Otto with the name of Theobald Smith. 22 guinea-pigs. *First injection*, horse serum ·002 to ·0025 c.c. with diphtheria toxin, $L+$dose, that is to say, the amount of diphtheria toxin which when mixed with one unit of standard antitoxin causes the death of a 250 gramme guinea-pig on the 4th or 5th day. *Second* injection, normal horse serum 6 c.c. *Interval*, $4\frac{1}{2}$ to 12 weeks. *Result*, 50 % of deaths.

Series II, also establishing the phenomenon. 14 guinea-pigs. *First injection*, horse serum ·235 to 6·4 c.c. with toxin $\frac{2}{3}$ to 19 times $L+$dose. *Second injection*, normal horse serum 6 c.c. *Interval*, 6 to 14 weeks. *Result*, all reacted, 6 died.

Series III, excluding sera other than horse serum. 16 guinea-pigs. *First injection*, horse serum with toxin. *Second injection*, rabbit serum 6 c.c. into 2; goat serum 10 c.c. into 11; ox serum 30 c.c. into 3. *Interval*, 5 to 10 weeks. *Result*, none reacted.

Series IV, excluding toxin. 34 guinea-pigs. *First injection*, toxin $\frac{1}{5}$ to $\frac{3}{5}$ $L+$dose. *Second injection*, normal horse serum 6 c.c. *Interval*, 4 to 11 weeks. *Result*, 32 gave no reaction, 2 died.

Series V, excluding toxone. 11 guinea-pigs. *First injection*, toxone. *Second injection*, normal horse serum. *Interval*, 6 to 10 weeks. *Result*, none reacted.

Series VI, isolating the specific agent. 32 guinea-pigs. *First injection*, normal or antidiphtheritic horse serum ·0025 to 10 c.c. *Second injection*, normal horse serum 6 to 7 c.c. *Interval*, $4\frac{1}{2}$ to 14 weeks. *Result*, guinea-pigs which had a large first dose showed no reaction. Guinea-pigs which had a small first dose reacted. None of series VI died.

There are clear deductions from Otto's experiments. Supersensitation is induced by horse serum. The reaction is specific for horse serum. Small first injections are more effective than large. Neither toxine nor toxone plays an essential part, though toxine appears to intensify. An interval of two weeks to two or three months must elapse between the injections. Otto determines further that the reaction is not due to precipitins for precipitins were not found.

Rosenau and Anderson (1906, p. 179) furnish details to show that the guinea-pig is more susceptible to two injections of the same serum than to two injections of sera derived from different species of animals. The phenomenon in their phrase is quantitatively and not absolutely specific. In other respects Rosenau and Anderson sustain Otto's deductions. They further suggest that supersensitation is induced in guinea-pigs by feeding with horse serum or with horse flesh, and that it is transmitted by the mother to her young. Anderson (1906, p. 259) in another paper records experimental evidence of this transmission.

It appears from the experiments of Otto and of Rosenau and Anderson that the repeated injection of guinea-pigs with horse serum induces a specific condition which is of the same order as the supersensitation effected by other extraneous sera and by bacterial products.

Corresponding phenomena in the human subject.

The repeated injection of horse serum for diphtheria of the human subject offers conditions analogous to those detailed for guinea-pigs. In the case of repeated injection of the human subject a fatal result is not reported by authors. Otto (p. 18) however, and von Pirquet and Schick (1905, p. 98) each record a case which came near to death, and Rolleston (1905, p. 664) refers to grave symptoms which may ensue within a few hours of injection in cases of relapse or of a second attack of diphtheria. Nevertheless while extreme severity is uncommon, the course of events which follows the administration to man of two suitable injections of horse serum differs in a more or less constant manner from the sequence after one administration.

The phenomena are minutely studied by von Pirquet and Schick (p. 98) in their work on the serum disease. Cases which react to two injections of serum are classed by these observers in three divisions: first, cases which show an immediate reaction only: second, cases which show an immediate reaction and an accelerated reaction: third, cases which show an accelerated reaction only. The immediate reaction is marked specifically by a local oedema of varying degree. The reaction is also attended by erythema, urticaria, constitutional disturbance and the like. It is apparent within 24 hours of injection. The accelerated reaction is described as a train of symptoms which differ from the results of a single injection in their earlier onset, briefer duration and frequently severer course.

Of 61 cases which are presented by von Pirquet and Schick in a tabular form as having received two injections of serum, 60 are suitable for classification in accordance with their predominant reaction.

First Division. Immediate reaction. 30 cases. Interval between injections of serum 12 to 50 days.

Second Division. Immediate reaction with accelerated reaction. 11 cases. Interval between injections of serum 2 to 6 months.

Third Division. Accelerated reaction. 19 cases. Interval between injections of serum 7 months to $7\frac{1}{2}$ years.

The *First Division* shows the immediate reaction in all cases. In four instances the accelerated reaction is also present, occurring for the first time when 21 days had elapsed between the first and the second injections. The *Second Division* with 11 cases shows the immediate reaction absent once and the accelerated reaction absent thrice. The *Third Division* with 19 cases shows the accelerated reaction absent once; the immediate reaction present on two occasions and certain cases doubtful. The divisions presented are not mutually exclusive nor are they precisely the divisions selected by the authors themselves, but they are based on the salient character of the reaction as defined by the interval between the first and second injections.

The first division begins with a minimum of twelve days' interval between the first and the second injections: with a shorter interval the result is negative: six cases injected at an interval of one day to six exhibited no reaction (p. 84). As with guinea-pigs, so with man, the interval between the injections of serum in the opinion of von Pirquet and Schick has an important influence in determining the presence and quality of the ensuing reaction. In the case of the immediate reaction in particular the details for guinea-pigs and for man show much agreement. The interval between injections for guinea-pigs is stated by Otto to be not less than two weeks, and up to two or three months. The interval for man is placed by von Pirquet and Schick between 12 days and six months: after this period the immediate reaction is rare. These authors agree with Otto in the view that the serum reaction has no essential relation to precipitins. In some of their instances precipitins were absent: in others they appeared at a different time from the serum reaction. Von Pirquet and Schick, in opposition to Otto and to Rosenau and Anderson, state that a large dose of serum at the first injection favours the immediate reaction.

Zucker (1905), in a series of 2323 cases, describes 24 which received serum for diphtheria on two or more occasions. He expresses the opinion that, whatsoever may be suggested by experiments on animals, the cases of his narrative offer no clinical indication of danger by a second or a third administration of antidiphtherial serum.

It is apparent from the observations cited that there occur in the human subject after two or more injections of horse serum, symptoms which, though much less severe, are comparable with the phenomena of supersensitation induced in guinea-pigs by similar treatment with the serum of animals of a different species.

Experience in Belvidere.

The persons who form the material of the Belvidere record were diphtheria patients unless otherwise described. A minority were contacts either with diphtheria or with bubonic plague. Serum administration was subcutaneous in most cases: in fewer instances the intravenous method was employed. The period included, beginning on 1st June 1901 and ending on 31st May 1906, shows a total of 168

persons who received injections of horse serum on more than one occasion. This total figure falls to 135 by the deduction of 33 cases of diphtheria which died within six days of admission, and which both in point of time and of severity of attack excluded the serum reaction. The 135 cases are thus constituted :—

Two injections	...	...	...	115
Three injections	...	...	...	18
Four injections	...	...	...	1
Five injections	...	...	...	1
				135

It is proposed to collate the above figures with a *General Return* of Belvidere cases injected with horse serum irrespective of the number of times during the approximately corresponding hospital period from 1st June 1901 to 30th September 1905. This General Return, which was prepared for a purpose unconnected with this note by the Superintendent (Brownlee, 1906) of these hospitals, yields 474 cases in all. The usefulness of the comparison resides in the circumstance that the same clinical material and the same sera are handled in both records.

Section A. Two Injections. Rash frequency.

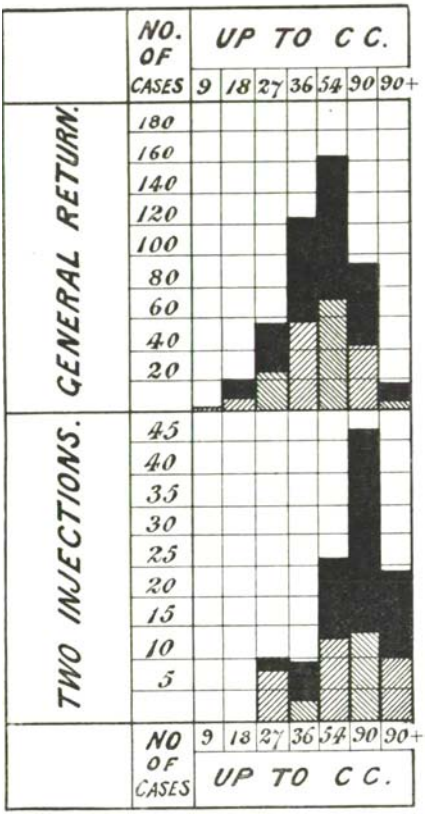
When the general return as shown in diagram A and table I. is compared with the twice injected in respect of the quantities of serum

TABLE I. *Number of Cases.*

	Up to	Rash	No rash	Total
General Return	9 c.c.	1	1	2
,,	18	12	8	20
,,	27	29	26	55
,,	36	64	58	122
,,	54	89	74	163
,,	90	52	42	94
,,	90+	12	6	18
Total		259	215	474
Two Injections	9	—	—	—
,,	18	—	—	—
,,	27	2	8	10
,,	36	6	3	9
,,	54	13	13	26
,,	90	33	14	47
,,	90+	13	10	23
Total		67	48	115

https://doi.org/10.1017/S002217240003312X Published online by Cambridge University Press

Diagram A.



given and without reference to the interval between administrations, it will be observed that the twice injected exhibit a higher dosage record than the cases of the general return. Evidence however of a correspondingly varying rash incidence is not provided under this section. The general return by table II. (p. 42) has a rash frequency of 54·6 %: the twice injected register a rash in 58·3 % of cases. The difference is not such as to form a basis for deduction.

With respect to rash frequency in the separate dosage groups the general return by table II. indicates a constant percentage from the group of 27 c.c. to the group of 90 c.c. where the numbers of cases are large enough to have significance. The figures of the other groups are too slight to found upon. A constant rash frequency with increasing dosage, which is contrary to the sense of most records, merits a reference in this place.

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TABLE II. *Percentage.*

	Up to	Rash	No rash	Tota
General Return	9 c.c.	50	50	100
,,	18	60	40	100
,,	27	52·7	47·3	100
,,	36	52·5	47·5	100
,,	54	54·6	45·4	100
,,	90	55·3	44·7	100
,,	90+	66·6̇	33·3̇	100
Total		54·6	45·4	100
Two Injections	9	—	—	—
,,	18	—	—	—
,,	27	20	80	100
,,	36	66·6̇	33·3̇	100
,,	54	50	50	100
,,	90	70·2	29·8	100
,,	90+	56·5	43·5	100
Total		58·3	41·7	100

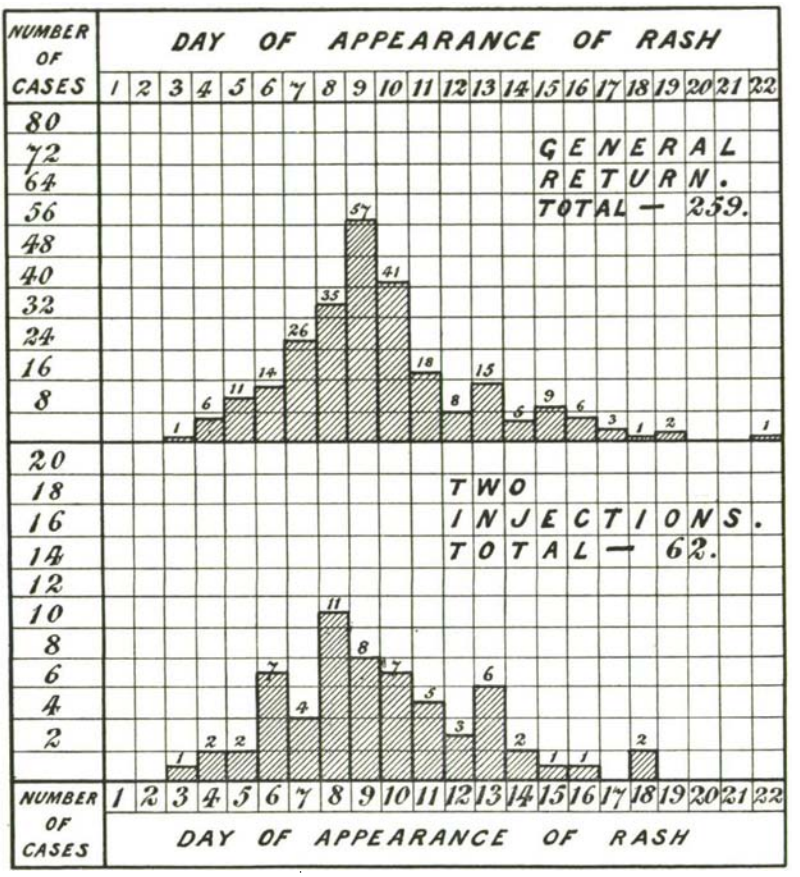
The twice injected on the other hand, fluctuating in the lower groups where the numbers are inconsiderable, present the relatively high rash frequency of 65·7 % in the 90 and 90+ groups together. The detail seems worthy of note. There is however no clear indication from the figures in general that a repeated dose of horse serum, without reference to the interval between injections, has an effect on rash frequency.

Section B. Two injections. Day of appearance of rash.

The time which elapsed between the injection of serum and the onset of rash in the general return is illustrated in the upper part of diagram B. The range of the incubation period is considerable, extending from the third day to the twenty second; but the majority of the rashes, 191 out of 259, have appeared by the end of the 10th day. A ten day interval thus seems a practical limit which may be applied to the twice injected cases in order to distinguish the influence of the first and second doses of serum respectively in producing rash. For the purpose of this section therefore the cases which received two injections are divided into two classes, first, cases which received the second injection of serum before the end of ten days counted from the first

injection, and, second, cases which received the second injection of serum after that period of time had elapsed.

Diagram B: up to ten days' interval.



When the general return is compared with the twice injected up to ten days' interval as indicated in diagram B it is noted that under the general return the maximum number of rashes, 57 in a total of 259, is recorded on the 9th day. Among the twice injected, on the other hand, up to ten days' interval, the 8th day has the maximum record, 11 in a total of 62. The crude maximum of the twice injected is thus earlier by one day. But the means of the two series are similar. The mean of the general return is 9·5; the twice injected up to ten days' interval

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have a mean of 9·4. The above considerations furnish no evidence that a repeated dose of horse serum accelerates the serum rash, when the interval between the first and second administrations is up to and not exceeding 10 days.

TABLE III. *Number of Cases up to ten days' interval.*

Days between S_1 and S_2	Day of appearance of Rash																	Total	Serial Number
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
½	S_1S_2			1		2		1		3			2	1				1	11	
1	S_1	S_2	1		1	3	1	4	3	2	2	1	2					1	21	
2	S_1		S_2	1	1	1	1	3	4		1	1				1			14	1
3	S_1			S_2			1	1	1	2		1		1	1				8	
4	S_1				S_2		1												1	
5	S_1					S_2 1		1											2	2, 3
6	S_1						S_2				1								1	
7	S_1							S_2 1											1	4
8	S_1																		—	
9	S_1									S_2	1								1	
10	S_1										S_2	1	1						2	5
Total			1	2	2	7	4	11	8	7	5	4	5	2	1	1		2	62	

S_1 = first injection of serum. S_2 = second injection of serum.

The total of table III. = 62, and the total of table IV. = 5, are together equal to 67, the total number of cases in table I. which showed a rash with two injections.

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The data which form the basis of the lower part of diagram B are detailed in table III. which shows for each case the day of appearance of the rash with reference to the interval of time between the injections of serum.

Although it has been stated above that the repeated injection of horse serum is not proved to accelerate the serum reaction when the interval between the injections of serum is ten days or a shorter period, it appears credible by reference to table III. that the administration of the second charge of serum towards the end of the incubation period of the first charge may suffice on occasion to determine the manifestation of a rash which would otherwise have remained undeveloped. The arrangement of the table furnishes graphic support for this suggestion. It will be observed that the line of second doses of serum in the table, receding from the line of first doses, excludes all rashes from the triangular area which is bounded by these lines and the base line. Case 3, which places its second injection in the first half of the latent period and which will be referred to immediately, is the only exception to this rule.

The suggestion is further sustained by the following consideration. In 7 of the 115 cases of table I. the second injection of serum took place in the latter half of the latent period, that is to say with 6 to 10 days' interval between the first and the second injections of serum. In two of these instances the rash failed to appear: five cases were positive as noted in table III. Though the numbers are slight the rash frequency, according to the standard of the cases reported in this paper, is high, and a rash when present invariably occurred after the second administration of serum.

Certain cases in table III. which are marked with a serial number may be briefly detailed.

Case 1. Ref. VI. 175, age 3½. Second injection of serum on 3rd day after first injection. Two rashes. First rash, marked in table, on 7th day from first injection of serum; urticarial and general. Duration 15 days with an interval. Second rash, not marked on table, on 34th day from first injection of serum; erythematous, mild. Duration 3 days. Considered as *without reference to repeated injection.*

Case 2. Ref. VII. 52, age 4. Second injection of serum on 6th day from first injection. Rash on same day as second injection, and immediately following second injection; urticarial, vivid. Duration five days. Suggested *determination of rash* by second injection of serum, in the sense that the rash in the absence of the second injection of serum might possibly have remained undeveloped.

Case 3. Ref. II. 151, age 1 7/12. Second injection on 6th day after first injection. First rash, not marked on table, on 4th day after first injection and before

second injection of serum. Erythematous, mild. Duration 1 day. Second rash, marked on table, on 3rd day from second injection of serum; urticarial, general, vivid. Duration 5 days. *Accelerated reaction.*

Case 4. Ref. VII. 38, age $2\frac{4}{12}$. Second injection of serum on 8th day after first injection. Rash on same day as second injection, and immediately following second injection. Urticarial, general. Duration 1 day. Suggested *determination of rash* by second injection of serum.

Case 5. Ref. Plague G, age 35. Second injection on 11th day. A plague contact. Yersin's serum 10 c.c. on both occasions. After first injection of serum, no symptoms. On the day following second injection, erythema and oedema of arm round puncture. *Immediate reaction.*

These five cases show the accelerated reaction once, the immediate reaction once, determination so-called in two instances, and one negative record.

TABLE IV. *Number of Cases over ten days' interval.*

Days between S_1 and S_2	Day of appearance of Rash										Total	Serial Number
	1	25	38	39	241	245	481	488	1001	1009		
24	S_1	S_2									—	6
37	S_1		S_2	1							1	7
240	S_1				S_2	1					1	8
480	S_1						S_2	1			1	9
1000	S_1								S_2	1	1	10
Total											4	

S_1 = first injection of serum. S_2 = second injection of serum.

The five cases of table IV. are also of the twice injected class, but they differ from the cases of table III. in that they received their second injection of serum after the lapse of a period of more than ten days counted from the first injection. Three cases of the five were without symptoms of supersensitation. One case at 37 days' interval exhibited the immediate reaction. One case at 240 days' interval showed the

accelerated reaction. It is indicated by the series in table IV. that the administration of the second of two injections of antidiphtherial serum after the close of the incubation period of the first, does not infallibly accelerate the rash or induce other signs of supersensitation.

The five cases of table IV. may be shortly recorded in this place under their serial numbers.

Case 6. Ref. XI. 10, age 6. Second injection of serum on 25th day after first injection. After first injection, rash on 14th day. After second injection, rash failed. Reaction of supersensitation *absent.*

Case 7. Ref. I. 194, age 6. Second injection on 38th day after first injection. Two rashes. First rash, not marked on table, on 7th day from first injection. Urticarial, general. Temperature 99·4°. Duration 5 days. Second rash on day after second injection. Erythematous, local. Temperature normal. Duration one day. *Immediate reaction.*

Case 8. Ref. III. 1, age 3. Second injection on 241st day after first injection. Two rashes. First rash, not marked on table, on 10th day from first injection; urticarial, mild. Temperature normal. Duration 5 days. Second rash on 5th day from second injection; urticarial, severe. Temperature 102·4°. Duration 11 days with an interval. *Accelerated reaction* with reinforcement.

Case 9. Ref. I. 1, age 4½. Two attacks of diphtheria. Second injection on 481st day from first injection. Rash not recorded after first injection. Rash on 8th day from second injection; urticarial. Temperature 99·4°. Duration not obtained. Reaction of supersensitation *absent.*

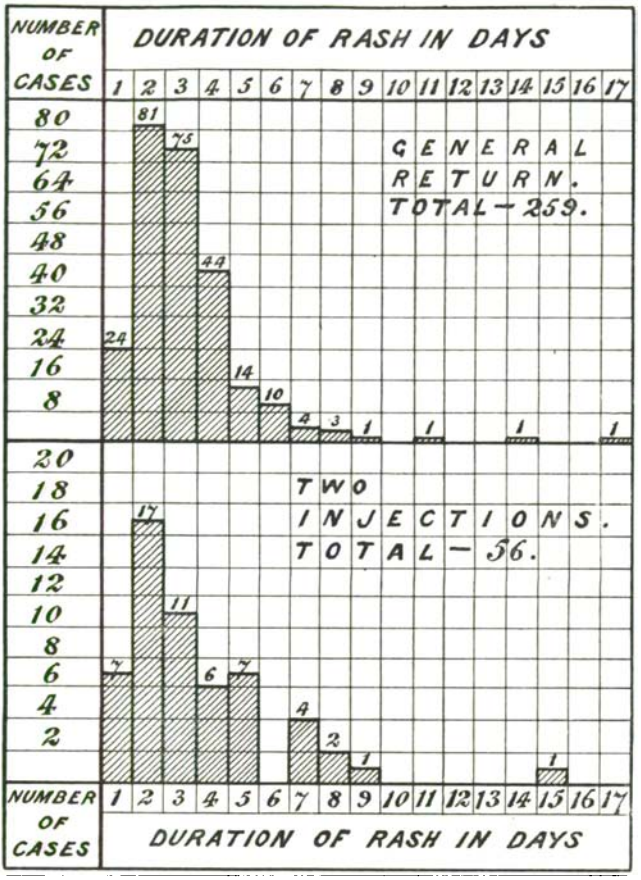
Case 10. Ref. Dr L, age 32. Second injection on 1001st day after first injection. First injection, 10 c.c. Yersin's plague serum for contact with plague. Second injection, 84 c.c. antidiphtheritic serum for diphtheria. After first injection no reaction. After second injection rash on 9th day; erythematous, local. Duration 2 days. Reaction of supersensitation *absent.*

Section C. Two injections. Duration of rash.

When the twice injected up to 10 days' interval, as in diagram C, are compared with the general return in respect of the duration of rash, the periods of 2 and of 3 days are observed to be the most common periods of duration in either class; and in general the two curves are similar. The mean of the general return is 3·3 days; the mean of the twice injected is 3·5 days. There is no proof that two injections under the conditions of this diagram influence the duration of the rash.

The details of the twice injected over ten days' interval may be briefly stated. The rash of case 6 lasted three days: of case 7, one day: of case 8, eleven days: of case 9, an unrecorded time: and of case 10, two days. The periods of duration vary to such an extent that no deduction seems possible.

Diagram C: up to ten days' interval.



The total for two injections of diagram C is less than the total for two injections of diagram B owing to the omission from diagram C of certain cases regarding the duration of whose rashes in formation was not obtained.

Section D. Three injections. Rash frequency and the like.

From table V which states the total quantities of serum received by the thrice injected it is apparent that this class represents a higher dosage rate than the general return or the twice injected. While the general return over 54 c.c. shows 112 cases in 474 a percentage of 23·6, and the twice injected in the same dosage groups have 70 cases in 115,

https://doi.org/10.1017/S002217240003312X Published online by Cambridge University Press

a percentage of 60·8; the thrice injected cases without exception are seen to have received more than 54 c.c. of serum. The thrice injected, which number 18, exhibit 13 rashes and 5 failures. The five cases which furnished no reaction had intervals of from 3 to 17 days between the first and the third administrations of serum.

TABLE V. *Dosage Rate.*

Up to	Rash	No rash	Total
54 c.c.	—	—	—
90	7	3	10
150	6	1	7
192	—	1	1
Total	13	5	18

The thrice injected are compared with other groups as regards rash frequency in table VI. According to this table the thrice injected have 72·2 % of rashes, a higher ratio than the preceding groups. The thrice injected are few, but an association between dosage and rash frequency is suggested.

TABLE VI. *Percentage comparison of thrice injected with the General Return and the twice injected.*

	Rash	No rash
General Return	54·6	45·4
Two Injections	58·3	41·7
Three Injections	72·2	27·8

Details regarding the thrice injected are presented in tabular form in table VII. The group may appropriately be divided into two series according as the last administration of serum falls within or without the ten day period to which reference was made above.

Series I. *Cases* 11 to 17 show the third injection of serum within the latent period: that is to say, not later than the 11th day. They do not differ essentially from cases of the general return. The severe reaction of *case* 11, ref. I. 149, age 4, on the 14th day from the first injection, is possibly worthy of remark: and notice may be taken of the late rash of *case* 17, ref. XI. 41, age 2, which appeared on the 25th day. Immediate and accelerated reactions are alike absent in this series.

Series II. *Cases* 18 to 23 show the third injection of serum without the ten days' interval.

Case 18. Ref. VI. 138, age 3. Total dose 135 c.c. The third injection of serum and rash both on 13th day, but rash preceding the third injection of serum. Possibly *accentuated reaction* in the sense that the reaction was more severe than might credibly have been the case in the absence of the third injection of serum.

TABLE VII. *Details.*

Serial Number	Day of administration of S_1	S_2	S_3	Days between S_1 and S_3	Day of appearance of rash	Duration of rash in days	Temperature	Quality of reaction
11	1	2	4	3	14	—	105·6°	Macular: vivid: general.
12	1	1	4	3½	9	3	99°	Urticarial: general.
13	1	2	6	5	8	5	103°	Urticarial: vivid: general.
14	1	2	8	7	9	2	Normal	Urticarial: moderate.
15	1	2	8	7	10	10	100°	Urticarial: recurrent.
16	1	3	10	9	15	2	Normal	Scarlatiniform.
17	1	8	11	10	25	2	102°	Morbilliform.
18	1	3	13	12	13	2	104·8°	Morbilliform: very vivid.
19	1	25	26	25	27	5	102°	Urticarial: general.
20	1	35	36	35	42	1	101·4°	Urticarial: local.
21	1	15	43	42	8	5	99°	Urticarial: general.
					45	1	Normal	Urticarial: local.
22	1	556	558	557	559	4	100·4°	Urticarial: general: very vivid.
23	1	1365	1838	1837	10	2	Normal	Urticarial: with articular pain.
					1372	10	Normal	Urticarial: severe.
					1846	1	Normal	Urticarial: extremely severe.

S_1=first injection of serum. S_2=second injection of serum. S_3=third injection of serum.

Case 19. Ref. II. 149, age 4. Total dose 126 c.c. Second injection of serum on 25th day. Third injection of serum on 26th day. Rash on 27th day. Probably *immediate reaction* after second injection.

Case 20. Ref. VII. 86, age $1\frac{2}{12}$. Total dose 72 c.c. Second injection on 35th day for a recrudescence of diphtheria. Third injection of serum on 36th day. Second injection followed in 7 days by rash of 1 day's duration. *Accelerated reaction* after the second injection suggested by brevity of rash.

Case 21. Ref. VII. 102, age 3. First injection 36 c.c. followed by first rash on 8th day. Second injection 27 c.c., for recrudescence of diphtheria, on 15th day, not followed by a rash. Third injection 27 c.c., for a further recrudescence, on 43rd day, followed in 2 days by second rash. First rash general, lasting 5 days. Second rash local, lasting one day. Second rash shows *accelerated reaction* in respect of early onset after third injection and in respect of briefer duration than the first rash.

Case 22. Ref. II. 50, age $2\frac{1}{2}$. First injection 12 c.c. prophylactic of diphtheria. No record of rash. Second injection 36 c.c. on 556th day. Third injection 21 c.c. on 558th day. Second injection followed in 3 days by rash, severe and of 4 days' duration. *Accelerated reaction* after second injection.

Case 23. Ref. Dr B. age 38. First injection Yersin's serum 10 c.c., prophylactic of plague, followed in ten days by first serum rash, urticarial with articular pain, of 2 days' duration. Second injection antidiphtheritic serum 18 c.c., for diphtheria, on 1365th day, followed in 7 days by the second serum rash, a severe urticaria of 10 days' duration. The second serum rash had thus a shorter latent period and a more rigorous course than the first serum rash. On 1824th day onset of influenza. On 1832nd day an urticarial rash not associated with serum, of moderate intensity and lasting 5 days. The third injection 27 c.c. antidiphtheritic serum for another attack of diphtheria, on the 1838th day, followed in 8 days by the third serum rash, an urticarial rash of extreme severity and of 1 day's duration. The third serum rash had a longer latent period than the second serum rash, a shorter than the first serum rash. Symptoms attending the third serum rash had violence and brief duration of *accelerated reaction*. The three rashes which ensued on the three administrations of serum show a progressive increase in severity. The occurrence of the rash not associated with serum suggests a constitutional facility as regards urticaria.

Series I. of this group, in which the third injection of serum falls within the latent period shows reactions which may be regarded as normal. Series II. of this group in which the third injection of serum falls without the latent period, has in each case a serum reaction which differs from the result of a single administration.

Section E. More than three injections.

This group contains two cases only.

Case 24. Ref. II. 104, age 6. 4 injections. The fourth injection of serum on 7th day. Total dose 198 c.c. Severe diphtheria. No rash. Death on 7th day from first injection of serum.

Case 25. Ref. IX. 126, age 7. 5 injections. The fifth injection of serum on 7th day. Total dose 222 c.c. Severe diphtheria. No rash. Recovery.

In each instance the last dose of serum fell within the latent period of the first, and the absence of exceptional reactions was to be expected.

General Considerations.

It is now proposed to compare the Belvidere cases with the cases recorded by von Pirquet and Schick: to state in a summary form the conclusions which the Belvidere observations suggest; and thereafter to note certain general aspects of the phenomena under discussion.

Experience at Belvidere is in accord for the most part with the results tabulated by von Pirquet and Schick (1905, p. 89) for double injection. The comparison may be extended also to the thrice injected cases of this paper.

The first division in the table of von Pirquet and Schick, which shows immediate reaction only, with a period of 12 to 50 days between the injections of serum, corresponds with cases 5, 7, and 19. Case 5 exhibits the immediate reaction with an interval of 10 days between the first and the second injections of serum, that is to say, with a shorter interval than is tabulated by von Pirquet and Schick for cases which furnished an unquestioned reaction. In this connection it may be borne in mind that, in the experience of the Belvidere Hospitals, the average incubation period of plague serum has been shorter by two days than the average incubation period of antidiphtheritic serum. Case 7 shows an immediate reaction when 37 days intervened between injections, and case 19 has an immediate reaction with a period of 24 days between the first and the second administrations of serum, a third injection on the day following the second injection being neglected. In Cases 7 and 19, therefore, the interval between the injections of serum falls within the 12 to 50 day period of von Pirquet and Schick's first division.

The second division of von Pirquet and Schick which showed both immediate reaction and accelerated reaction, with an interval

of two to six months between injections, is not represented in this record.

The third division of von Pirquet and Schick which displayed the accelerated reaction only, and in which a period of from seven months to seven and a half years elapsed between the two injections of serum, may be compared with the following cases.

Case 3, which showed a first rash on the 4th day from the first injection of serum, had the second injection on the 6th day from the first injection. The second injection was followed in two days by the second rash, an example of the accelerated reaction. A five days' interval between two injections which induced an accelerated reaction is a lower record than is furnished by von Pirquet and Schick's table, where the accelerated reaction—with an immediate reaction preceding—appears for the first time with a period of 21 days between two injections of serum.

In case 20 the accelerated reaction occurred with an interval of 34 days between the first and second injections of serum, a third injection on the day following the second injection being neglected. Case 21, which exhibited a first rash on the 8th day from the first injection of serum, had on the 15th day a second injection which was not followed by a rash. The third injection in this case, which was administered on the 43rd day from the first injection, induced a rash which had the accelerated character. Cases 20 and 21, therefore, with periods of 34 and 42 days respectively between the first and the effective subsequent injection of serum, have shorter intervals between injections than the cases of von Pirquet and Schick's third division,—whose minimum interval between injections is seven months,—and may be compared with instances in their first or second divisions, where the accelerated reaction occurs with or without a preceding immediate reaction, and where the interval between injections of serum is up to and not exceeding six months.

In case 8 the accelerated reaction was noted, the interval between the first and the second injections of serum being 240 days. Case 22 exhibited the accelerated reaction with a period of 555 days between the first and the second injections, a third injection two days after the second injection being neglected. In case 23, 1364 days intervened between the first and the second injections, and 1837 days between the first injection and the third. In this case an accelerated reaction was observed both after the second and after the third injections. Cases 8, 22 and 23, therefore, as regards length of interval between injections,

are within the period of von Pirquet and Schick's third division, which extends from seven months to seven and a half years.

Certain statements are suggested by a view of the Belvidere cases.

The Twice Injected. There is no evidence that the twice injected have a higher *rash frequency* than the general return.

There is no evidence that the *latent period* among the twice injected, when the interval between the first and second injections is up to 10 days, differs from the latent period of the general return. It is credible that the administration of the second injection of serum in the closing days of the latent period of the first should on occasion make manifest a rash which would otherwise have failed to appear The administration of the second of two injections, after the termination of the latent period of the first, may curtail the latent period of the second injection, but is not infallible in this respect.

There is no evidence that the rash among the twice injected differs in *duration* from the rash of the general return.

The Thrice Injected. The thrice injected show a higher *rash frequency* than the general return or the twice injected. This is coincident, and probably associated with a relatively higher dosage rate.

There is no evidence that the *latent period* among the thrice injected, when the interval between the first and the third injections is up to and not exceeding 10 days, differs from the latent period of the general return. When the interval between the first and the third injections is over 10 days, the thrice injected consistently show a modification of the ensuing serum reaction with a reduction in the length of the corresponding latent period.

From the foregoing statements it emerges that the interval of time between the first and the second injections of serum is a primary factor in determining the abnormal reaction which has been regarded as evidence of supersensitation. The length of the interval is at least more essential to the phenomenon than the administration of serum in large doses. Von Pirquet and Schick concur in this opinion, even although they differ from other observers in considering large doses more effective than small.

It will be recalled that Wright (1903) has drawn attention to processes in immunization where the interval between administrations of the active substance has an important influence. He indicates, for example, that the inoculation of vaccine is followed in the first place

by a diminution, and in the second place by an increase in the bactericidal value of the blood. The fall and the subsequent rise he terms the negative and the positive phases of the immunity curve. He states that a cumulative negative phase is produced by injection of the immunizing agent during the negative phase of the preceding administration, and that a cumulative effect is most to be feared when the amount injected is excessive. Supersensitation, however, does not appear as a cumulative result of excessive dosage. Otto (p. 16) found small quantities of serum more disastrous to guinea-pigs than large: he is followed by Rosenau and Anderson (1906), who procured supersensitation of a guinea-pig by $\frac{1}{1{,}000{,}000}$ c.c. of horse serum, a dose whose minuteness recalls the report of von Behring and Kitashima (1901, p. 162), that the death of an animal was caused by successive injections of diphtheria toxin which amounted in sum to $\frac{1}{400}$th of the minimum lethal dose. In view of such data as these, the cumulative theory seems inapplicable to the phenomena of supersensitation.

Certain other theories which are mentioned by authors (see von Pirquet and Schick, 1906) may be noted in this place. Courmont's absorption theory would explain supersensitation on the hypothesis that the first injection of the active material leads to the absorption of a natural protective substance, and that the animal is thus left defenceless against the second injection. Bail has expressed the view that death, after two injections of serum, is associated with the production of a substance which impedes the activity of leucocytes. Richet has suggested that the phenomena in question are due to the presence in the serum injected of two separate bodies, of which one causes immunity and the other supersensitation. It is a disadvantage of the theories of Courmont, Bail and Richet that they are formulated without special regard to the influence exerted by the interval of time between the separate administrations of serum.

The endotoxin theory of Wolff (1904) has reference to the effects produced in the animal tissues by the liberation of poisons contained within the bodies of bacteria, whose external covering has been penetrated or dissolved as a result of defensive measures adopted by the animal concerned. In the opinion of Wolff there is no immunity to foreign albumens of the endotoxin class: but, even if it be the case that the action of endotoxins furnishes an adequate explanation of abnormal phenomena which may follow the introduction into an animal of bacteria whose hurtful effect is exerted after the destruction of their

outer covering, the theory does not seem applicable to extraneous blood sera, whose constituents are free at the time of injection.

The precipitin theory is discussed by Otto, by Rosenau and Anderson, and by von Pirquet and Schick. According to these observers the presence of precipitins in the blood of animals or human beings, whether normal or supersensitized, did not synchronize with the serum reaction. In some cases the serum reaction was the first to appear: in others, precipitins were earlier recorded. In some cases, precipitins were present, and the serum reaction was absent: in others, the reverse occurred. Nevertheless, it is noteworthy that precipitin formation and the serum reaction, whether typical or abnormal, tend to be manifest side by side, as if they resulted by the action of allied substances.

That these substances are probably antibodies is suggested by the following considerations. In the case of *precipitin*, it was shown by von Dungern (1903), who experimented on rabbits with the plasma of the crustacean *Maja squinado*, that the latent period before the appearance of precipitin constantly approximated to six days: similarly, the *serum* reaction, as is matter of common knowledge, develops after an interval of incubation. Further, in the case of *precipitin* it is recorded by von Dungern (*ibid.*), in the first place, that rabbits immunized to the plasma of *Maja squinado* show precipitin after a shorter latent period than normal rabbits; and, in the second place, that repeated injection of rabbits with maja plasma within the latent period of six days does not accelerate the appearance of precipitin: similarly, in the case of *serum* phenomena it is the sense of this note and of the works quoted, in the first place, that the sensitized organism furnishes an earlier reaction than the organism not previously treated, and, in the second place, that the administration of succeeding injections of serum within the incubation period of the first fails to accelerate the ensuing rash. The serum reaction not less than precipitin formation is in accord with the law of the latent period, which concerns the development of antibodies. The occurrence of the serum reaction after the injection of extraneous sera may thus be regarded as due to the elaboration of antibodies in the organism, by substances in the injected material which are allied but not identical with the substances which induce precipitin formation.

The *normal* serum reaction, in accordance with this theory, will be understood in the sense that the injection of extraneous serum leads after a quiescent interval to the elaboration of antibody, and that by

the interaction of a substance contained in the serum injected and of the antibody which it originates, there is formed a toxic product whose presence is made known by the so-called serum reaction. It is credible from analogy that the antibody in question is gradually brought into existence: it may be, therefore, that the antibody-producing substance, uniting with nascent antibody, gradually frees the toxic product, which the organism under these circumstances is able gradually to eliminate. Or, it is possible that the toxic product evokes a secondary antibody, which combines with the toxic product, controls its effects, and ultimately brings the visible reaction to an end. The incubation period of this secondary antibody must be regarded as shorter than the incubation period of the primary antibody.

In the case of the *abnormal* serum phenomena which mark the supersensitive state, there is support for the opinion that the immediate and the accelerated forms are to be ascribed to different causes.

The immediate reaction is exempt from the law of the latent period. If the antibody has a part in the phenomenon, it is not necessary at least that time should elapse in order that the antibody may be prepared. On the contrary, it is to be supposed that the antibody to the first injection of serum is still present when the second injection is given. The antibody-producing substance of the second injection of serum, and the antibody already produced by the first injection, come in contact, under these circumstances, without delay: their union is rapid: the whole charge of the poisonous substance is freed in a brief period, and the toxic symptoms tend to be sudden and severe. If the theory of a secondary antibody is accepted as relevant in respect of the normal serum reaction, the special characters of the immediate form of the abnormal reaction will be explained on the view that, while the primary antibody produced by the first injection of serum persists at the time of the second injection of serum, the secondary antibody evoked by the toxic substance which resulted from the combination of the first injection of serum with the primary antibody has already vanished from the organism. The disappearance of the secondary antibody within a limit of days may be supported on the analogy of relapsing fever, in which Löventhal[11] records a rapid fall of the bactericidal content of the serum during the apyrexial interval of the disease. When, therefore, the antibody-producing substance of the second injection of serum reacts with the primary antibody produced in the organism by the first injection of serum, the abruptly liberated toxic product of this reaction exerts its hurtful influence unchecked

until sufficient time has elapsed to admit of the preparation anew of a secondary antibody to control its effects. When the immediate reaction is absent, it is open to belief that the primary antibody has disappeared, or was present in too small a measure to induce an appreciable result.

With regard to the general mechanism of the processes considered, it may be, in terms of Ehrlich's hypothesis[9], that the second administration of serum takes place at a time when specific side chains, elicited by the first injection of serum, are still in course of formation, and that the higher affinity of the so-called sessile receptors for antibody-producing substances contained in the second injection is responsible for the distinctive features of the immediate serum reaction.

The accelerated reaction differs from the immediate reaction in observing the law of the latent period. The accelerated reaction occurs with or without a preceding immediate reaction, and may be regarded as independent of the presence of antibody in the animal at the time of the second or succeeding injection of which it is the result. Rather it is to be attributed to a tissue modification, in virtue of which there is developed a more rapid cellular response to the stimulus of material present in the serum injected. The accelerated reaction is evidence of the acquisition on the part of the animal of a faculty which is normally useful. Certain parasitic diseases in nature, which obtain a foothold in the animal tissues, attack these through the natural channels of access, and by means of small quantities of bacteria, living organisms which are capable of growth and reproduction. The multiplication of the infecting bacteria in such cases may be taken generally as a sign that their assault is being attended by a measure of success. An animal, therefore, which developes the capacity of rapidly preparing antibodies to restrict the proliferation of such noxious agents, when introduced through natural channels, has achieved an immunity to parasitic diseases of a certain natural type.

But, if the active principle is introduced into the system neither through the customary channels nor under the form of a micro-organism, whose power for mischief depends on its liberty to grow and multiply, the procedure is out of accord with the course of nature, and the defensive powers of the animal, adapted to cope with natural infections, are somewhat at fault in their method of dealing with the artificial invasion. It is true that a mechanism of the protective class is stimulated by repeated injections of extraneous serum into an animal;

but the protective value of the mechanism in question resides in the circumstance that it is suited to check the elaboration of toxic products within the system; it is not adjusted to neutralize or counteract a definite dose of such a substance as blood serum, which is not capable of numerical increase. Extraneous sera appear to belong to an order of substances which effect immunization, not by inducing insusceptibility of the tissue cells, but by means of an accelerated reaction, which may thus be regarded as the expression of a misdirected defence, a formal but useless immunity.

The accelerated reaction conforms to the theory that two antibodies are concerned in serum phenomena. To the more rapid formation of the primary antibody, the reduction of the latent period may be looked on as due: to the speedier preparation of the secondary antibody the accelerated reaction owes its commonly briefer course.

Other aspects of the serum reaction which are discussed or disputed by authors have been noticed in their place. The capricious variability of the signs under conditions apparently similar, the relative effectiveness of large and of small first doses, the comparative mildness in man of the immediate reaction, the apparently exclusive incidence in man of the accelerated reaction, and the possible sensitizing influence on man of a diet of horse-flesh are problems of interest, but their solution is not promoted by the data of this paper. The relationship, also, of intravenous injection to supersensitation remains open to discussion.

From the practical standpoint, however, it is apparent that the facts detailed are consistent with the view that repeated injections of horse serum induce symptoms of supersensitation in man; but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of antidiphtherial serum. Experiments on animals may favour the opinion that such a disaster is a speculative possibility in the case of the human subject, but there is no reported evidence to the effect that it is so imminent a danger as to excuse a restricted application of the antitoxin treatment.

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