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THE IMPORTANCE OF METALLIC SALTS FOR AMBOCEPTOR FORMATION

The following is the translation of an article by A. F. Walbum and S. Schmidt in the German-language periodical Immunitaets Forschung (Immunity Research), Vol 43, 1925, pages 32-437

Success has been achieved, as is well known, in influencing the concentration of antibodies in an actively immunized organism by means of various chemical actions. Jorgensen and Madsen (1) brought about an increase in diphtheria antitoxin in the case of diphtheria-immunized horses by an injection of Pilocarpin. Roux and Vaillard (2), Jorgensen and Madsen (3), Friedberger and Dorner (4), Schroeder (5), Pfeiffer (6), Reymann (7), and others showed that one large bloodletting or several small ones can stimulate to antitoxin formation. Similar shifting in antibody production can be effected by the injection of Pyrodin and Pyrogallol (Madsen and Tallquist,8), Metol (Mueller,9), Methylene blue (Pucrat,10), Salvarsan (Walker,11), and thorium (Hektoen and Corper, 12). Recently Walbum (13) and Walbum and Jørch (14), have studied metal salts more closely with reference to their importance for antibody formation and their influence on this and have found that there are several among these substances

which even have an unusually universal action in this respect. Ehrlich, who began these experiments on the basis of various theoretical considerations, reports as follows on the matter:

"With our present knowledge, it is difficult to go more closely into the question of the character of the processes which go on in the organism during the formation of antitoxin but of what a type the processes may be(synthetic processes, splits, intramolecular rearrangements, etc.), one may probably assume that transformations of an enzymatic nature play a more or less outstanding role here as in the case of all cell activity.

Certain conditions, as is well known, must be present so that the enzymes may be able to display their full activity, conditions, concerning the nature of which, our knowledge is very slight. Among other things, one has observed that the presence of certain metallic salts can exert a considerable and sometimes a decisive influence (catalyzers, co-enzymes) on many enzyme actions (several oxydases), which leads me to suspect that certain metallic salts might perhaps also be of importance for the antitoxin-forming processes in the animal organism, and -- in case this proved to be correct -- that the type and amount of those sorts of salts in the organism could perhaps be one of the causes of the often considerable individual differences of the antitoxin formation ability of the animals. It would be to be supposed that every animal that is at all able to react to a toxin injection by the formation of an antitoxin possesses the necessary enzymes (specific?) for such a reaction which thus

become decisive for the qualitative relations during antitoxin formation while the type and amount of the present metallic salts (catalyzers?) more or less determine the quantitative conditions of these processes.

In case the state of affairs were thus, one might assume that an addition of the metallic salt concerned to the organism that is being immunized would, in many instances, increase the scope of the antitoxin-formation process, that is, would have to be expressed in an increase in the antitoxin concentration of the blood."

The experiments first undertaken referred to the action of manganese chloride, nickel chloride, cobalt chloride, and zinc chloride on the formation of the diphtheria antitoxin in actively immunized horses and on the Coli-agglutinin formation in goats. The injections were undertaken intravenously and it was shown that all the tested salts resulted in an increase in antibody production, although in a differing degree.

Horses immunized against diphtheria which showed a steady increase in the strength of the antitoxin could, in several cases, by the injection of manganese chloride, be brought to an antitoxin concentration which was greater than that which had been originally achieved by the usual immunization with toxin alone. At the Statens Serum Institute, injections of manganese chloride have been introduced into immunisation technology in the production of diphtheria antitoxin and other serums.

Walbum and March (14) who continued and further investigated these experiments showed, among other things, that it is possible in this way also in the case of diphtheria-immunized goats to push the antitoxin concentration of the blood considerably higher than is usually possible by toxin treatment alone, a fact which can claim a certain practical interest. For the immunization of human beings it is often desirable to be able to use goat diphtheria antitoxin; however, up to now, it has usually been impossible by the usual immunization to bring the antitoxin in the blood of a goat higher than to about 60-80 immunizing units per cubic centimeter, an antitoxin strength which is pretty low for practical use. We succeeded in our experiments in producing a serum with manganese chloride that contained 165 immunizing units per cubic centimeter.

The injected manganese chloride disappears very rapidly from the blood circulation and almost the entire amount of manganese given off from the experimental animal is removed through the intestinal mucous membrane. Not a small part is retained in various organs. However the ability of these to store manganese differs greatly. Usually the manganese content of the organs increases gradually with the amount injected. However, in this connection, the liver forms an exception.

It seems that the ability of this organ to retain manganese stands in a certain relationship to the antitoxin production capacity of the animal so that good antitoxin producers have an increased manganese content in the liver while the

amount in the liver of poor antitoxin producers is considerably reduced. This manganese depot in the liver of the animal is perhaps of significance for antitoxin formation (catalyzers?).

To compare the action of different metallic salts, extensive experiments were undertaken with Coli-immunized animals (goats and rabbits). In this connection it was shown that the action of various metallic salts differs exceedingly. In agreement between the action of the metals and their place in the periodic system does not seem to exist. On the other hand, if one arranges them according to the somewhat older viewpoints, one will find that within groups so set up there is, in most cases, an express agreement between the atomic number and the action in this way that the action within individual groups (alkaline metals? and the calcium group) increases as the atomic number increases while within other groups the opposite relation prevails (magnesium group, silver group, iron group? and perhaps the platinum group).

It was found out in addition that beryllium chloride is the most active of all the salts included in the experiment.

Since it is obvious that these relations, in addition to the purely practical interest, can claim no small interest from the standpoint of the immunity theory, there is an intention of testing gradually the importance of the metal salts for other processes in the organism which have greater or slighter direct connection with the science of immunity and, in the first place, to ascertain whether the peculiar depend-

ence of the action on the atomic numbers of the substances is a law with general application within this area.

Thus Walbum (15) has tested the action of various metallic salts on the bactericide substances of the blood and found that the degree of activity of these is also influenced in several instances and in no small degree by the metallic salts just as a certain agreement is found here too between the atomic number and the action.

In the present work we have examined the importance of metallic salts for amboceptor formation.

The technology applied was as follows:

Defibrinated sheep blood /Hammelblut/ was centrifuged by shaking and the blood corpuscles separated off by centrifuging were washed three times by means of a sodium chloride solution. One part of these washed blood corpuscles was dissolved in two parts of sterile distilled water and 3 cubic centimeters of this solution(= 1 cubic centimeter of blood corpuscles) were injected intravenously into the experimental animal(rabbit). Before the injection a blood sample was taken to see whether normally occurring amboceptors were present in this, which was in no case true in the animals used.

The injection of the blood corpuscle solution usually, at any rate apparently, produced no symptoms of poisoning in the rabbits. From the 5th day after the injection blood samples were taken daily and on the 16th day after the injection on which the amboceptor curve would supposedly be descending sharply, one cubic centimeter of a 0.001 molar solution of the metallic

salt in question (in a physiological sodium chloride solution) was injected intravenously per kilogram of the rabbit. According to the experiments of Kalbum and Mørch this is the largest amount of the most powerfully toxic metal salt(cadmium), which one can inject intravenously in a rabbit without producing visible symptoms of poisoning.

Blood samples were taken 10 minutes, 30 minutes', 1, 2, 3, 5, 7, and 24 hours after the metallic salt injection. The measurement of the amboceptor concentration of these serum samples was carried out as follows:

At first a preliminary experiment was conducted. The serum was diluted 1 : 250 and from this dilution dosages 1.0-0.3-0.1-0.06-0.03-0.01 were used, together with a reliably solvent complement dosage (twice the amount of the dosage found solvent by the experiment) and 0.25 cubic centimeter of a 5-percent deposit [Aufschwemmung] of sheep blood corpuscles in a total volume of 1.25 cubic centimeters. After careful shaking, the glasses were placed for one hour in a 37-degree water bath, then shaken again, and stored until the next day in an ice cellar [Eiskeller] at a temperature of about 2 degrees.

The reading of the experiment was taken in this way: By means of a glass with a 30-percent hemolysis a comparison of all the series was so conducted that the amount was determined which produced such a hemolysis for each serum sample. The reciprocal value of this amount showed the number of amboceptor units per cubic centimeter of undiluted serum. The main experiment was of course conducted with far smaller doses in the

dosages than in the indicated preliminary experiment.

The action of the individual metallic salt injection was determined by the height to which it could drive the amboceptor concentration of the serum and in relation to the maximum amboceptor concentration achieved in advance through the antigen alone. The indicated percent increase is to be judged in relation to the first acme of the curve while this is put at 100. In this way individual differences of the individual experimental animals regarding antibody formation are eliminated--insofar as this is at all possible -- and the results attained are thus directly comparable with one another.

Let it be noted here that only those rabbits were used for the experiments which showed a vigorous amboceptor formation after the blood injection since it turned out that animals which showed a sluggish amboceptor formation also reacted weakly to the metallic salt injection. Among the rabbits whose amboceptor formation was good, there were, however, some (about 10-15 percent of the animals used) that did not react at all to the following metallic salt injection. These animals we have of course disregarded.

In the included Table I all the results of these experiments are cited while the metals are arranged according to their action. Three rabbits were used for each metal. Of course not all of the test results are cited but only the few which are of importance in this connection. In the last column of the table the average percent increase occasioned by the metallic

salt injection is cited.

Induced by some experiments of Walbum(16), according to which very small amounts of various metallic salts often exert a furthering action on the formation of bacterial toxins in the cultures, Helena Purdy and Walbum (17) undertook a series of experiments on the action of metallic salts on the course of various hemolytic processes among others also on the complement-amboceptor-hemolysis (sheep blood). Since it was proved, in this connection, that many of these salts, in minute amounts, exerted a furthering or checking action on the hemolysis, one could fear that the amounts of metallic salts which were transmitted to the hemolytic system with the rabbit serum were so large that they interfered with the reaction. By simple calculation, however, one sees easily that the amounts which can be transmitted in this manner are so small that they lie far below the border of activity for the various metallic salts.

Table I

Salt	First Acme	Amboceptor Units Per Cu Cm Before Injection of Salt	Second Acme	Increase in %	Average Increase
$MnCl_2$	1920	833	1470	33	28
	1470	1000	1470	32	
	1430	714	1000	20	
$PbCl_2$	2500	1250	1920	27	24
	3850	3000	4050	27	
	1000	455	630	17.5	
$BaCl_2$	1670	556	1090	32	27
	1460	1000	1430	29	
	1250	833	1110	22.2	
$MgCl_2$	385	192	217	6.5	20
	250	135	217	32.5	
	217	111	156	20	
$BaCl_2$	2780	1920	2780	30	20
	455	313	357	10	
	1000	500	714	21	
$MgCl_2$	417	167	313	35	18
	1250	625	769	11.5	
	200	110	125	7.5	
$SrCl_2$	3130	1470	2170	22	16
	625	250	313	10	
	3850	2500	3130	16.5	
$CaCl_2$	2500	1670	2220	22	15
	2500	1330	1670	14	
	3850	2860	3210	9	
AgCl	3850	3130	3850	18	14
	2500	1000	1370	15	
	833	435	500	7.8	
$CdCl_2$	556	278	345	12	13
	278	147	192	16	
	1000	385	500	11.5	
$CrCl_3$	500	278	400	24	13
	1920	1470	1670	10	
	3130	1470	1670	6	
$CuCl_2$	1000	833	909	7.6	12
	3850	1220	2000	20	
	313	125	147	7	

Table I

Salt	First Acme	Amboceptor Units Per Cu Cm Before Injection of Salt	Second Acme	Increase in %	Average Increase
$ZnCl_2$	1250	1090	1250	13	11
	909	556	625	8	
	625	426	500	12	
$OsCl_4$	1250	1250	1470	17	9
	909	125	155	3	
	1670	714	833	7	
$CoCl_2$	556	250	313	11	7
	833	385	435	6	
	833	313	350	4.5	
$NiCl_2$	1470	625	700	5	7
	2500	1470	1670	8	
	1920	833	1000	8.6	
$FeCl_3$	2780	1670	1920	9	7
	5560	3130	3450	5.75	
	1110	630	709	7	
$HAuCl_4$	1470	1000	1110	7	7
	1920	1470	1590	6	
	833	500	556	6.7	
H_2PtCl_6	1250	500	556	4.5	4
	1250	714	769	4.4	
	833	435	455	2.4	
$AlCl_3$	250	192	192	0	0
	2500	1920	1920	0	
	1000	500	500	0	

In Table II the metals are arranged by groups according to their chemical properties and within the individual groups according to their atomic numbers. In the third, fourth, and fifth columns respectively there is cited the action of the salts on amboceptor formation, agglutinin formation and on the bactericide substances occurring in plasma. It may be concluded from this that for amboceptor formation too a certain relationship exists between the stimulating action of metals and their atomic numbers.

Table II

	Atomic Number	Amboceptor	Agglutinin	Bactericide Substances
		Magnesium Group		
Be	9.1	27	1147	63.0
Mg	24.3	18	565	4.2
Cu	63.6	12	334	1.7
Zn	65.4	11	188	1.3
Cd	112.4	13	44	1.2
(Hg)	200.6	20	181	
		Calcium(cerium)Group		
Ca	40.1	15	27	0.44
Sr	87.6	16	43	0.26
Ba	137.4	20	233	0
Pb	207.2	24	402	0
		Silver Group		
Cu	63.6	12	334	1.7
Ag	107.9	14	95	1.3
Au	197.2	7	30	0.83
		Iron Group		
Co	59.0	7	20	5.6
Ni	58.7	7	30	0.71
Mn	54.9	28	142	0.44
Fe	55.8	7	0	0.13
Cr	52.0	13	48	0.67
		Platinum Group		
Os	190.1	9	268	0.59
Pt	195.2	4	36	0.87
Al	27.1	0	166	0

In the magnesium group the action decreases from beryllium to zinc whereupon it rises again to cadmium and quicksilver.

In the calcium group the action decreases steadily as the atomic number rises while the behaviour of the silver group is apparently the reverse.

Within the iron group there can probably only be talk of a comparison between the three bivalents cobalt, nickel, and manganese and it seems as if an increased action occurs as the atomic number decreases.

If one investigates within different groups the action of the individual metallic salts on the amboceptor formation, agglutinin formation, and the bactericide substances of the blood, then one will obtain the following results.

In the magnesium group the action of the metallic salts on agglutinin formation and on the bactericide substances decreases as the atomic number rises and conditions regarding amboceptor formation are apparently partially (or perhaps completely) of the same type. The numbers for copper, zinc, and cadmium (12, 11, and 13) lie so close to one another that any difference certainly lies within an experimental error.

A uniformity likewise exists in the silver group in that the action here too decreases as the atomic number rises.

Within the calcium group conditions are the same in the case of amboceptor and agglutinin formation in that the action increases as the atomic number increases while the bactericide substances behave in an opposite manner.

An agreement exists also in the iron group between amboceptor and agglutinin formation. Here, however, the action increases as the atomic number decreases and here too the bactericide substances behave in just the opposite way.

In regard to the two trivalents, iron and chromium, the action of chromium is greatest in all investigated cases.

In regard to the platinum metals osmium acts with more powerful stimulation than platinum on the formation of amboceptors and agglutinins while action, in the case of the bactericide substances, apparently proceeds in the opposite direction.

Any attempt wishing to explain these phenomena theoretically now seems to us to be premature.

As is shown by Walbum and Mørch(14), the concentration of the injected metallic salt plays an extraordinarily important part in its degree of action, since there seems to be a best concentration and the action of both greater and lesser concentrations seems to decrease.

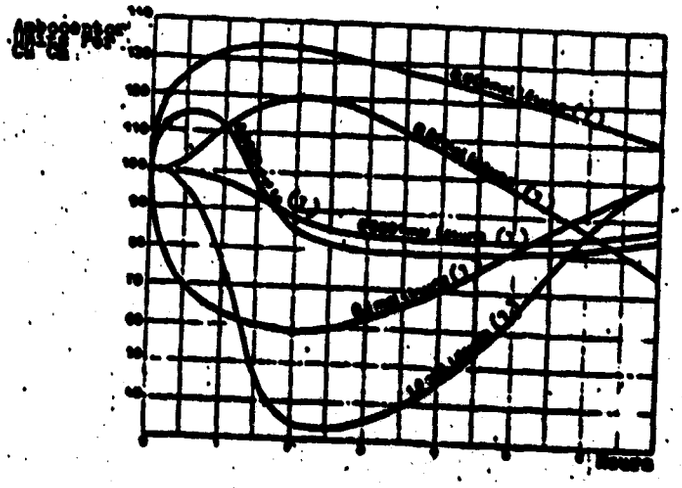
The greatest effect on agglutinin formation was attained by means of a 0.001 molar solution.

We have tested this relation in regard to amboceptor formation by injecting beryllium chloride solutions in the indicated concentrations into a series of rabbits whose amboceptor curve was falling steeply. Blood sampling took place before the injection and 10 minutes, 2 hours, and 7 hours after the injection. In table III the result of the experiment is cited in such a way that the numbers indicate the number of amboceptor units per one cubic centimeter of serum. The graphic presentation is found

Table III

Amboceptor Units Per Cu Cm

$BeCl_2$ Molar Solution	Before Injection	10 Minutes After Injection	2 hours After Injection	7 hours After Injection	Dead
1.0	100	100	33	100	After 15 hrs
0.1	100	77	59	100	After 18 hrs
0.01	100	100	120	75	
0.001	100	120	133	110	
0.0003	100	113	85	85	
0.0001	100	100	87	87	



Legend:

(1) Molar solution

From this it may be concluded that while 1.0 and 0.1 molar solutions cause a sharp drop in the amboceptor concentration of the blood, the 0.01, 0.001, and 0.0003 molar solutions cause rises, while the greater dilution, a 0.0001 molar solution, results most quickly in a slight drop.

Just as for agglutinin formation, the greatest effect seems to be produced here by an approximately 0.001 molar solution.

Summary

~~This~~ IT article, in which the influence of various metallic salts on the amboceptor formation of the rabbit is examined, is a link in ~~the constantly continued~~ A CONTINUING research PROGRAM on the action of metallic salts on the immunological and other processes in the animal organism. ~~Just as in works which appeared earlier, the authors find here too~~ IT WAS CONFIRMED THAT within the individual groups a certain agreement among the atomic numbers of the metals and their action.

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