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The Feasibility of Demonstration of GM- and InV-Systems in Decaying Organs

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Summary. γ-globulin factors (Gm and InV-systems) were determined in 12 cadavers in blood and various organ exprimates (kidney, liver, spleen and muscle). Also checked was the time interval up to which these factors could still be demonstrated in decaying organs.
For this purpose blood and portions of the organs were left to decay in plastic containers at an average temperature of 19.1°C. Examinations were done with the agglutination-inhibition-test. The exprimates were employed in dilutions 1:10 and 1:20. The serum factors Gm (1), (2), (4), (10) and InV (1) could be demonstrated for varying periods of time.
In organs they could be demonstrated for between one and eight weeks. Only factors Gm(1) and Gm(2) could be demonstrated beyond this time. As a rule these serum factors were demonstrable in blood for a longer period of time than in organs. Factor Gm(4) proved to be the most stable one, as it could be demonstrated up to 11 weeks in decaying serum.
In 2200 individual tests with a dilution 1:20 no false positive results were obtained, with the dilution 1:10, however, 5 false positive tests were found.
No explanation can be given for the different periods of time for which these factors can be demonstrated under conditions of decay.

Zusammenfassung. Bei zwölf Leichen wurden im Blut und in verschiedenen Organpreßsäften (Niere, Leber, Milz und Muskel) die γ-Globulineigenschaften (Gm- und InV-Systeme) bestimmt und ihre Nachweiszeit bei Fäulnis überprüft. Blut und Organteile wurden hierbei in verschließbaren Plastikgefäßen bei einer mittleren Temperatur von 19,1°C faulen gelassen. Die Untersuchungen erfolgten mit dem Agglutinationshemmtest.
Die Preß- bzw. Fäulnissäfte wurden in 1:10 und 1:20 Verdünnungen verwendet. Die Serumfaktoren Gm(1), (2), (4), (10) und Inv(1) konnten über unterschiedlich lange Zeiträume nachgewiesen werden.
In den Organen betrug die Nachweiszeit zwischen einer und acht Wochen, wobei nur die Faktoren Gm(1) und Gm(2) über diese Wochen nachweisbar blieben. Die Serumeigenschaften waren im Blut allgemein länger nachweisbar als in den Organen. Am stabilsten erwies sich der Faktor Gm(4), der bis zu elf Wochen im faulenden Serum festgestellt werden konnte.

Bei 2200 Einzelbestimmungen wurden mit der 1:20 Verdünnung in keinem Fall falsch positive Ergebnisse erzielt, mit der 1:10 Verdünnung hingegen fünf falsch positive Ergebnisse.
Eine Erklärung für die unterschiedlich langen Nachweiszeiten unter Fäulnisbedingungen kann nicht gegeben werden.

Key words. Gm-system, in decaying organs – In V-system, in decaying organs – Decaying organs, Gm- and InV-systems

Introduction

The hereditary properties of γ-globulins (Gm and InV-systems) are employed among others for identification of tissues and blood traces. Since only very small amounts of material are required they have achieved considerable importance in the examination of biologic traces.

The possibility to demonstrate Gm-serum factors in dried spots of blood extends, according to statements published up to now, to a period of time from 3 months to 16 years (Ducos et al. 1962, Heifer and Haupt 1963, Prokop and Byrdy 1965).

Abe and Nakajima (1974) were able to demonstrate InV-properties up to 3 months, Blanc (1973) was even able to demonstrate InV-properties in blood stains from donors with known phenotypes that had been stored for as long as 22 years. When the blood stains were stored in a moist environment the period of demonstration for Gm-factors extended only up to 5 or 6 weeks (Nielsen and Henningsen 1963, Lenoir and Muller 1966).

Gramer (1975) succeeded in demonstrating Gm-factors (1), (2), (4), (10) and InV (1) in blood samples that had been stored for 3 to 4 years under refrigerator conditions, here however Gm (1) in one case and Gm (10) in two cases could no longer be demonstrated.

Kijewski (1972) spilled drops of blood in various types of soil. She obtained positive results from sand and garden soil after 20 days, with vegetable soil and loam this was possible up to 8 days whereas clay and calcareous soil yielded the „worst" results, no exact data were given here. Blood with the properties Gm (1+), (2–), InV (1–) gave false positive results following concentration of the samples irrespective of the type of soil.

Krämer (1963) determined Gm (1) in human tissues (liver, spleen, kidney, brain, muscle, periostium, epithelial squames, hairs, finger nails, cornea) and in body fluids. The tissue studies here were done both in the fresh (washed and unwashed) state and after mumification by 8 days of dehydration in the air of an incubator. Gm (1) could be demonstrated in the mumified state too in all tissue samples with capillaries, but not in tissues without capillaries.

Oepen (1972) reported results of determination of Gm (1), Gm (2) and InV in tissue extracts of skin, muscle, spleen and kidney. She obtained a number of wrong results already in „fresh" organ extracts. In tissue samples „aged" for 2 weeks on the other hand no wrong results were described (conditions of storage not stated).

In a homicide case 4 years ago Gm and InV-systems among others were of great importance for identification of cadaver parts. Different results were obtained by 2 experts who had determined Gm-factors from decaying organs. As is evident from the review of the literature no statement can be made regarding the period of possible

determination of Gm and InV-factors in decaying cadaver parts, an investigation thus appeared necessary.

We were interested in particular for how long the serum properties Gm (1), (2), (4) and (10) and InV (1) can be demonstrated in decaying blood and in decaying organs.

Methods and Material

Portions of myocardium, kidney, spleen, liver and blood were obtained at autopsies from 12 bodies 24 to 60 hours post mortem (Table 1).

Tissue fluid for the determinations was obtained by „squeezing" the „fresh" organs with broad anatomic forceps.

Parts of these organs were kept in closed plastic flasks and left to decay at temperatures between 16 and 22°C, mean 19.1°. Fluid from the decaying tissues for further determination was pipetted from these flasks after additional squeezing.

Methods for Determination of Gm and InV

Gm and InV-factors were determined by agglutination-inhibitiontest.

In order to sensitize the erythrocytes, fresh erythrocytes of blood group 0; CcDEe, ccDEE or CCDee and M and P negative were coated with incomplete anti-rhesus-sera.

The erythrocytes of the blood groups required were obtained in part by DADE corporation, some were (also) prepared by 2 workers of our department.

The sensitization of erythrocytes that were to be used for determination of Gm-factors (1), (4) and (10) was done according to the procedures given by Molter corporation. For these determinations anti-rhesus- and anti-Gm-sera produced by the same company were also employed.

Sensitization for Gm-factor (2) was done according to the formula of ASID company whose sera were employed.

Sera or tissue fluid and decay fluid, respectively, were used diluted with (physiological NaCl solution) 1:10 and 1:20.

Examinations

1. Gm-factors (1), (2), (4) and (10), as well as InV(1)-factor were determined immediately in blood serum and fluid of the „fresh" organs.

Table 1. The following serum factors were present in the cadavers

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
cadaver 1 (GS 122/75)	+	–	+	–	–
cadaver 2 (GS 124/75)	+	–	+	–	–
cadaver 3 (GS 125/75)	+	–	+	–	–
cadaver 4 (VS 102/75)	+	–	+	+	–
cadaver 5 (VS 104/75)	+	–	+	+	–
cadaver 6 (VS 105/75)	–	–	+	+	–
cadaver 7 (VS 106/75)	+	–	+	+	–
cadaver 8 (GS 136/75)	+	+	+	+	+
cadaver 9 (GS 137/75)	–	–	+	+	–
cadaver 10 (VS 111/75)	–	–	+	+	–
cadaver 11 (GS 150/75)	+	+	+	+	–
cadaver 12 (VS 112/75)	+	+	+	+	+

2. Control tests of Gm-factors and InV-factors were then done over a total period of 4 months every 6–8 days.

3. At every other examination we checked by means of the Ouchterlony-test whether the amounts of substrate necessary for determination of Gm and InV were still present in the decaying organs. Ouchterlony-tests were done on commercially available immuno-agar-plates of Heyland company. The fluids from decaying organs were diluted 1:10 with physiologic NaCl as prescribed and then allowed to diffuse against the anti-serum in a series of geometric dilutions. Results were read after 24 hours.

4. In addition smears for bacteriologic studies were taken from the decaying organs of 5 cadavers up to the end of our studies. These smears were inoculated at the Department of Hygiene and Microbiology into endo- and blood plates as well as nutritiant broth.

Together with each determination the following controls were done:
1. Check of sensitization of erythrocytes.
2. Check for antibodies.
3. Check for hemolytic factors.
4. Check of the antiserum.
5. Positive and negative control sera were run parallel with each test.

Results

Serum Factor Gm (1):

In 2 out of 9 cases there was a discrepancy regarding the demonstrability in serum and tissue exprimate (kidney, spleen, liver and muscle) at the time of the first determination. Gm(1)-factor was once found in the serum, whereas it was not possible to demonstrate it in exprimates of the organs. In the second case the factor was found in the exprimates, but not in the serum.

In both cases the examination 6 days later showed concordance, i.e. Gm(1)-factor could be demonstrated both in serum and in fluid from the decaying organs.

Gm(1) was demonstrable in serum and exprimates of the decaying organs for the following periods of time (X = 1 case):

	1 week	2 weeks	3 weeks	4 weeks	5 weeks	8 weeks	10 weeks
serum	X	X	XX	X		XX	XX
kidney	X	XXXX		XX		XX	
spleen	X	XX		XX	XXXX		
liver	XXX	XXXX		X	X		
muscle	X	XXX	XX	XXX			

Serum Factor Gm(2):

At the time of first determination serum factor Gm(2) could be demonstrated in 3 cases in both serum and organ exprimates for the following periods of time (X = 1 case):

	2 wks	3 wks	4 wks	5 wks	6 wks	7 wks	8 wks	9 wks	10 wks
serum							X	X	X
kidney			X				XX		
spleen		X				X	X		
liver	X	X	X						
muscle		X		X		X			

Serum Factor Gm(4):

Serum factor Gm(4) could be demonstrated in all 12 cadavers. In one case there was a discrepancy at the first determination: whereas the factor could be demonstrated in all organ exprimates, it was not possible to do so with serum.

The tests done one week later showed Gm(4) both in serum and in exprimates of the decaying organs.

Gm(4) could be demonstrated in serum and exprimates from decaying organs for the following periods of time (X = 1 case):

	3 wks	4 wks	5 wks	6 wks	7 wks	8 wks	9 wks	10 wks	11 wks
serum		X		X	XXX	XXXX	XX		X
kidney	XXX	XXXX	X	X	XXX				
spleen	XXXX	XXX	XXXX	X					
liver		XXXXX	XXXX	XX					
muscle	XXXXX	XXX							

Serum Factor Gm(10):

Serum factor Gm(10) was present in 9 cases. In three cadavers the factor could not be demonstrated in one organ during the whole period of time examined (kidney, liver and muscle respectively), whereas it was demonstrable in serum and the 3 other organs for a certain period of time.

Gm(10) could be demonstrated for the following time (X = 1 case):

	1 week	2 weeks	3 weeks	4 weeks	5 weeks
serum	X		XX	XXXX	XX
kidney	XXXXX	XX			
spleen	XXXXXXXXX				
liver	XXXXXXX	X			
muscle	XXXXXXXX				

Serum Factor InV(1):

In 2 cadavers the serum factor InV(1) was present (Table 1). In 1 cadaver InV(1)-factor could be demonstrated neither in exprimates from fresh nor from decaying muscle, whereas it was demonstrable for one week in serum, kidney, spleen and liver. In the other cadaver, demonstration was successful in serum for 6 weeks, for 1 week in the kidney and for 2 weeks in spleen, liver and muscle.

Ouchterlony-Test:

The Ouchterlony-test done on immuno-agar-plates from the beginning to the end of the study for all factors showed in all cases precipitations and thus „sufficient amounts of substrate" (Schwerd 1974).

Bacteriologic Studies

Bacteriologic studies in the decaying organs yielded the following strains of bacteria:

Grampositive Cocci	Gramnegative Enterobacteria	
staphylococcus albus	escherichia coli	aerobic spore
streptococcus viridans	klebsiella	forming organisms
enterococci	proteus mirabilis	
(strept. faecalis)	pseudomonas	

No relationship was recognizable between „time of decay" and bacterial growth or „time of decay" and alterations of bacterial growth.

Because of the few number of experiments *statistics* have not been performed.

Discussion

The present results indicate, that Gm-factors (1), (2), (4) and (10) and InV(1)-factor are demonstrable in decaying blood and organs for varying periods of time (Tables 2–5).

The average period of time for which Gm(1)-factor can be demonstrated is about 5 weeks in serum, in kidney and spleen about 4 weeks, in liver about 2 weeks and in muscle about 3 weeks. For Gm(2)-factor these periods were about 9 weeks in serum, about 7 weeks in the kidney, about 6 weeks in spleen, about 3 weeks in liver and about 5 weeks in muscle.

For Gm(4)-factor the average times were about 8 weeks in serum, about 5 weeks in kidney, about 4 weeks in spleen, about 5 weeks in liver and about 4 weeks in muscle. For Gm(10)-factor the average times were some 4 weeks in serum, in the organs kidney, spleen, liver and muscle about 1 week.

InV(1)-factor was present in only 2 cadavers, no mean values therefore were calculated.

In general all factors were demonstrable in serum for longer periods of time than in the organs.

Factor Gm(4) could be demonstrated for the longest period of time, namely 11 weeks in decaying serum.

No explanation can be given for the varying periods of time for which these serum factors can be demonstrated. These time intervals present an indicator for the stability of these factors.

That these factors can be demonstrated in decaying serum and organs, respectively for only comparatively short periods of time in comparison with the considerably longer intervals within which it is possible to demonstrate them in dried traces of blood (Prokop and Byrdy 1965, Blanc and Gortz 1973, Ducos et al. 1962, Gramer 1975) is probably due to alterations going along with decay. In dried blood or in samples stored in the refrigerator bacterial contamination probably is much less and therefore demonstration of Gm and InV-factors is possible for longer periods of time. The higher the degree of temperature and humidity as well as of bacterial contamination the faster both autolysis and decay will proceed which decreases the length of time for which these factors can be demonstrated.

Fünfhausen (1962), both Nielsen and Henningsen (1963) and Lenoir and Muller (1966) pointed out, that humidity has a very adverse effect upon the conservation of blood traces. They could no longer demonstrate Gm-factors after moist storage for 5 or 6 weeks.

Table 2. Length of time for which Gm and InV-factors could be demonstrated

cadaver 1:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	2 weeks	not present factor	6 weeks	not present factor	not present factor
kidney	4 weeks*)		4 weeks*)		
spleen	4 weeks		6 weeks		
liver	4 weeks		6 weeks		
muscle	2 weeks		3 weeks*)		

cadaver 2:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	4 weeks	not present factor	9 weeks	not present factor	not present factor
kidney	2 weeks		7 weeks		
spleen	4 weeks		4 weeks		
liver	2 weeks		6 weeks		
muscle	3 weeks*)		3 weeks*)		

cadaver 3:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	3 weeks	not present factor	4 weeks	not present factor	not present factor
kidney	2 weeks*)		3 weeks*)		
spleen	2 weeks		3 weeks		
liver	2 weeks		4 weeks		
muscle	2 weeks		3 weeks		

* In control tests for hemolysing factors hemolysis occurred. No further determinations were done since controls were not possible any more.

Table 3. Length of time for which Gm and InV-factors could be demonstrated

cadaver 4:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	8 weeks	not present factor	8 weeks	4 weeks	not present factor
kidney	4 weeks*)		4 weeks*)	2 weeks	
spleen	5 weeks		5 weeks	1 week	
liver	5 weeks		4 weeks	2 weeks	
muscle	4 weeks*)		4 weeks*)	1 week	

cadaver 5:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	1 week	not present factor	8 weeks	1 week	not present factor
kidney	1 week		3 weeks	1 week	
spleen	1 week		4 weeks	1 week	
liver	1 week		5 weeks	1 week	
muscle	1 week		5 weeks		

cadaver 6:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	not present factor	not present factor	7 weeks	3 weeks	not present factor
kidney			4 weeks*)	1 week	
spleen			5 weeks*)	1 week	
liver			4 weeks		
muscle			3 weeks*)	1 week	

* In control tests for hemolysing factors hemolysis occurred. No further determinations were done since controls were not possible any more.

Table 4. Length of time for which Gm and InV-factors could be demonstrated

cadaver 7:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	8 weeks	factor not present	7 weeks	5 weeks	factor not present
kidney	2 weeks		3 weeks*)	1 week	
spleen	5 weeks		3 weeks	1 week	
liver	2 weeks		4 weeks	1 week	
muscle	3 weeks		4 weeks*)	1 week	

cadaver 8:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	3 weeks	9 weeks	9 weeks	4 weeks	6 weeks
kidney	2 weeks	4 weeks	5 weeks	1 week	1 week
spleen	2 weeks	3 weeks	3 weeks	1 week	2 weeks
liver	1 week	3 weeks	4 weeks	1 week	2 weeks
muscle	2 weeks	3 weeks*)	3 weeks*)	1 week	2 weeks

cadaver 9:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	factor not present	factor not present	7 weeks	4 weeks	factor not present
kidney			6 weeks		
spleen			3 weeks	1 week	
liver			4 weeks	1 week	
muscle			3 weeks	1 week	

* In control tests for hemolysing factors hemolysis occurred. No further determinations were done since controls were not possible any more.

Table 5. Length of time for which Gm and InV-factors could be demonstrated

cadaver 10:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	not present factor	not present factor	11 weeks	5 weeks	not present factor
kidney			4 weeks	2 weeks	
spleen			5 weeks	1 week	
liver			5 weeks	1 week	
muscle			3 weeks*)	1 week	

cadaver 11:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	10 weeks	10 weeks	8 weeks	4 weeks	not present factor
kidney	8 weeks	8 weeks	7 weeks	1 week	
spleen	5 weeks	8 weeks	4 weeks	1 week	
liver	1 week	2 weeks	5 weeks	1 week	
muscle	4 weeks	5 weeks*)	5 weeks*)	1 week	

cadaver 12:

	Gm(1)	Gm(2)	Gm(4)	Gm(10)	InV(1)
serum	10 weeks	8 weeks	8 weeks	3 weeks	1 week
kidney	8 weeks	8 weeks	7 weeks	1 week	1 week
spleen	5 weeks	7 weeks	5 weeks	1 week	1 week
liver	2 weeks	4 weeks	5 weeks	1 week	1 week
muscle	4 weeks	7 weeks*)	4 weeks	1 week	

* In control tests for hemolysing factors hemolysis occurred. No further determinations were done since controls were not possible any more.

It is to be assumed, that autolysis or decay result in alterations of the protein structure of Gm and InV-factors. The Ouchterlony-tests that weere positive in all studies do not contradict this.

Unlike the Gm-agglutination-inhibition-test blood which presents an antigen-antibody-reaction of a selective kind, the antibodies employed in the Ouchterlony-test react against a variety of proteins or „fragments" of proteins, i.e. the Ouchterlony-test presents a polyvalent reaction.

Effects of bacteria on the demonstrability of the serum factors, as they were discussed by Kijewski (1972) as possible explanation of her false positive results, could not be ascertained, at least not for the bacterial strains found by us in the decaying organs.

Gm(1)-factor could be demonstrated for the longest period of time in organs of 2 cadavers, in each of which proteus mirabilis was found bacteriologically. A pure culture therefore was inoculated into serum in order to check a possible influence on the result of the test. No changes of Gm and InV-factors however were observed after incubation for 50 hours. With a dilution of 1:10 one false positive result each in 5 cadavers was obtained out of a total of 2200 individual tests. A factor that could be demonstrated neither in serum nor organ exprimates at the time of the first examination was later found positive in one organ. *False positive* results were obtained for factor Gm(10) three times, for factor Gm(2) and InV(1) one time each.

At a dilution of 1:20 at which also 2200 individual tests were done, on the other hand no false positive results were found.

Five false results at a dilution of 1:10 correspond, related to 2200 individual tests done, to a percentage of some 0.2 %. Whether certain products of decomposition of proteins result in a blockade of antibodies via adhesion or some other causes are responsible for these false positives has to remain open. The "zero failure rate" at a dilution 1:20 is possibly due to the "per se" higher dilution of the sample extract and the resulting decrease of blockade since antibody and antigen are in a more favourable relation to each other.

Chemical effects as cause of the false positive results can be excluded, since the material examined was treated uniformly.

The role of chemical effects is described by Blanc, Gortz, Ducos and Madrarge (1971). They obtained for example a false positive result when checking for Gm(1) in blood traces on a coat hanger that had been treated during manufacture with ortho-chlorophenol.

False negative results in the first determinations are due to the method of obtaining organ exprimates. Little or no serum at all could be obtained by squeezing "fresh" material with forceps. For subsequent determintation, however, sufficient amounts of serum could be obtained with the same technique due to autolytic changes in the organs.

We can state as the result of our studies, that demonstration of serum factors in decaying organ material is possible only up to a certain period of time that is subject to individual variations.

When no serum factors can be demonstrated in decaying organs this does not mean that this factor was nor present originally, a fact that has to be taken into consideration in stating opinions in forensic cases.

On the other hand it can be said, that if a serum factor is positively demonstrated this factor has actually been present provided both a dilution 1:10 and 1:20 had been used for the agglutination-inhibition-test.

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