EFFECT OF HEAT AND ULTRA-VIOLET RADIATION ON ISO-AGGLUTININS IN DRIED BLOOD STAINS

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The primary object of the present study was to see to what extent adverse conditions would affect the grouping of bloods by means of dried stains kept in circumstances similar to those likely to be met with in ordinary medico-legal practice. For this we chose to deal with the iso-agglutinins as being more labile than the agglutinogens, and to expose the stains to dry heat and to ultra-violet radiation—these being factors likely to occur in practice, particularly in the Tropics—the influence of which on the stain might be important.

Method

Blood was collected from eighteen volunteers of both sexes and allowed to dry at room temperature on glass slides, on scraps of leather, and soaked up in ordinary plain surgical gauze. The material was kept in unsealed envelopes lying on the laboratory bench, conditions being thus pretty much what might obtain in the case of a stain met with in practical medico-legal work. The agglutinins of the stains were tested against cells of Type A, Type B, and two specimens of Type O, designated O1 and O2 respectively. The cells were collected in citrated saline, washed once, and suspended in 0.85 per cent. saline in a 3 per cent. suspension. The bulk of the tests were carried out on blood dried on slides, but no differences were detected in stains on leather or gauze. In each case a fragment of stain was detached and placed on a slide, a small quantity of dilute cell suspension added, and a cover-glass applied. After five minutes the cover was very gently agitated and the slide examined for agglutination, examination being again made at the end of a further five minutes. Brisk stirring is to be avoided, as it greatly encourages rouleau formation. The blood group of the donor had been determined at the time of collection of the blood stain by the usual slide method.

Results

All specimens were examined ten days after collection and without any further treatment. In each case the group was determined without difficulty and correctly, the worker reading the result being of course kept in ignorance of the already determined group. The results are tabulated in Table I.

One batch of six stains was heated to 64° C. for one hour in an electric drying oven and then submitted to grouping as before. Little, if any, diminution in agglutinin content was noticed, no difficulty being experienced in obtaining a correct group in each case at the first attempt.

Portions of all stains were then heated to 100° C. for one hour in the drying oven and typing done as before. The results are shown in Table III.

A further typing was carried out on a group of stains similarly treated six days after, the results of which experiment are listed in Table IV.

Difficulty was experienced in diagnosing the group of some of the stains, mainly in those cases where too brisk stirring had been employed. In all cases, so far as could be judged, the strength of the agglutinins was considerably diminished, and larger portions of the stains had to be taken to get agglutination.

Portions of seven stains (two from each of Groups A, B, and O, and one AB specimen) were placed in small glass staining blocks and exposed for one hour to ultra-violet light at fifteen inches from a Hanovia quartz analytical lamp. Care was taken to see that the portions of dried clot did not overlap and screen

TABLE I.—*Stains Untreated*

Group already Deter- mined	No. of Speci- men	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
A	1	—	+	—	—	A
A	2	—	+	—	—	A
O	3	+	+	—	—	O
O	4	+	+	—	—	O
B	5	+	—	—	—	B
A	6	—	+	—	—	A
O	17	+	+	—	—	O
O	10	+	+	—	—	O
A	13	—	+	—	—	A
B	12	+	—	—	—	B
A	9	—	+	—	—	A
O	7	+	+	—	—	O
O	11	+	+	—	—	O
A	16	—	+	—	—	A
O	14	+	+	—	—	O
A	15	—	+	—	—	A
AB	18	—	—	—	—	AB
A	8	—	+	—	—	A

TABLE II.—*Stains Heated for One Hour at 64° C.*

Group already Deter- mined	No. of Speci- men	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
A	1	—	+	—	—(R)	A
A	2	—	+	—	—(R)	A
O	3	+	+	—(R)	—(R)	O
O	4	+	+	—	—	O
B	5	+	—	—	—	B
A	6	—	+	—	—	A

(R) indicates rouleau formation

TABLE III.—*Stains Heated for One Hour at 100° C.*

Group already Deter- mined	No. of Speci- men	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
O	3	+	+	—	—	O
O	11	+	+	—	—	O
O	10	+	+	—	—(R)	O
O	14	+(R)	+	—(R)	—	? O
O	17	+	+	—	—	O
A	6	—	+	—	—	A
A	13	—	—(R)	—	—	? AB
A	9	—	+	—	—	A
O	7	+	+	—	—(R)	O
AB	18	—(R)	—(R)	—	—	? AB
B	12	+(R)	—(R)	—	—	? B
A	8	—	+	—	—	A
A	15	—(R)	+	—	—	? A
A	16	—	—	—	—	AB
A	1	—(R)	+	—	—	? A
B	5	+	—	—	—	B
A	2	—(R)	+	—	—	? A
O	4	+	+	—	—	O

(R) indicates rouleau formation.

TABLE IV.—*Stains Heated for One Hour at 100° C.; Second Batch*

Group already Determined	No. of Specimens	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
O	10	+	+	—	—	O
AB	18	—	—	—	—(R)	AB
O	7	+	—	—	—	B
A	16	—	+	—	—	A
A	8	—	+	—	—	A
A	15	—	+	—	—	A
O	4	+	+	—	—	O
A	13	—	—	—	—	AB
O	17	+	+	—	—	O
A	1	—	+	—	—(R)	A

(R) indicates rouleau formation.

each other from the rays. The stains were afterwards grouped, and so far as could be observed the radiation produced no effect. A further experiment was therefore carried out. Portions of all stains were taken three weeks after bleeding and exposed in a similar fashion for three hours without apparent effect.

The effect of heat combined with ultra-violet light was investigated in a further group of six stains by exposing for one

TABLE V.—*Stains Exposed Three Hours to Ultra-violet Light*

Group already Determined	No. of Specimens	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
O	11	+	+	—	—	O
O	3	+	+	—	—	O
A	16	—	+	—	—	A
B	12	+	—	—	—	B
B	5	+	—	—	—	B
A	8	—	+	—	—	A
A	2	—	+	—	—	A
O	17	+	+	—	—	O
A	6	—	+	—	—	A
O	10	+	+	—	—	O
O	7	+	+	—	—	O
A	1	—	+	—	—	A
O	14	+	+	—	—	O
AB	18	—	—	—	—	AB
A	15	—	+	—	—	A
A	13	—	+	—	—	A
A	9	—	+	—	—	A
O	4	+	+	—	—	O

TABLE VI.—*Stains Heated One Hour at 100° C. and then Exposed One Hour to Ultra-violet Light*

Group already Determined	No. of Specimens	Agglutination of Cells				Group Diagnosed
		A	B	O1	O2	
O	10	+	+	—	—	O
AB	18	—	—	—	—	AB
O	3	+	+	—	—	O
A	1	—	+	—	—	A
A	2	? +	+	—	—	? O
O	7	+	+	—	—	O

hour at fifteen inches from the quartz lamp stains which had previously been subjected to dry heat for one hour at 100° C. The results are contained in Table VI.

It will be seen that in most cases radiation has produced no further effect on the agglutination except in Specimen 2, which shows a doubtful positive agglutination of A cells. The main thing to be noticed is the absence of any trouble from rouleau formation.

Discussion

It is evident that drying at room temperature and storage for ten days in ordinary conditions does not seriously affect the readiness with which stains can be grouped by their agglutinins. These also appear to resist dry heat for one hour at 64° C. without serious impairment, but a similar exposure to 100° C. does cause some loss of power. A further point is the apparent increase of the factors tending to regular rouleau formation. This may be apparent only, and due entirely to the extra concentration necessary to produce agglutination; but our feeling is that this is not the whole explanation, since we noticed the appearance of this phenomenon when the concentration of the stain was not greatly increased. It is curious also that subsequent irradiation seems to diminish rouleau formation almost to vanishing-point. To investigate these facts further is not possible by the relatively crude methods we have used in the present series of experiments.

Conclusions

1. In agreement with generally accepted views no difficulty has been experienced in accurately diagnosing the group of a blood by the agglutinins present in a dried stain. Moreover, no difficulty ensues after exposing the stains to dry heat at 64° C.

2. Dry heat at 100° C. diminishes the strength of the agglutinin factor to a considerable extent, but does not usually abolish it completely; and this temperature seems to have some influence on the appearance of regular rouleau formation.

3. Ultra-violet radiation under the above-described conditions produces no obvious effect on the agglutinins. On the other hand, it does seem to have a certain influence in diminishing the formation of regular rouleaux.

4. It seems unlikely that ordinary blood stains would lose much of their agglutinin content if kept dry, even if they were exposed to strong sunlight and fairly high temperatures.

BIBLIOGRAPHY

The literature on the subject is so extensive that no attempt is made to cite it here.

So far our search has not shown any work on the agglutinin factors in dried stains on the lines of the present investigation. A good review of the general technique of blood grouping is to be found in a paper by J. C. Thomas (*Medico-Legal and Criminological Review*, 1937, 5, 54).

The National Institute of Industrial Psychology (Aldwych House, W.C.2) has just published the July-August issue of *The Human Factor*, price 2s. 6d. The first article, by Mr. H. D. Henderson, deals with the displacement of labour by machinery. Mr. T. G. Rose, recently appointed general director of the Institute, has written an article on clearer business vision, in which he stresses the need for good will in any industrial concern. Dr. O. A. Oeser of St. Andrews University deals with the complex social and psychological factors which may determine the choice of a career. The Institute is now developing an advisory service to educational authorities on the design and provision of equipment, and on methods of heating, lighting, and ventilation in schools. Results of various investigations are detailed in an article on school buildings.