DETERMINATION OF RED CELL ISOENZYME GROUPS OF THE PHOSPHOGLYCOLATE PHOSPHATASE (PGP) AND THE PROPERDIN GROUPS (Bf) IN STORED SAMPLES OF BLOOD, HEMOLYSATE AND SERUM UNDER VARIOUS CONDITIONS.

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It is desirable to know under which storage conditions later investigations of blood samples will be sucessful,e.g. for a control investigation of known samples or to disprove a presumed mix-up of samples drawn for a blood alcohol test. These questions can also become important for those cases where blood samples have to be investigated that were stored or transported for some time under unfavourable circumstances.

In order to obtain informations concerning these problems for the red cell isoenzyme of the phosphoglycolate phosphatase (PGP) and the properdin factor B (Bf) we determined the groups of these two systems in samples which were stored under various conditions.

For the determinations the following storage conditions were chosen:

1. Preparation of the hemolysates, immediately after the blood was drawn or after a one-night storage at $4^{o}C$ and storage afterwards at $-18^{o}C$.
2. Preparation of the hemolysates after keeping the blood samples at $4^{o}C$ for 3 days and storage afterwards at $-18^{o}C$.
3. Preparation of the hemolysates after first keeping the blood samples at room temperature for 3 days then at $4^{o}C$ for 8 days and finally storage at $-18^{o}C$.
4. Preparation of the hemolysates immediately after the blood samples were drawn or sent in and storage afterwards at $-40^{o}C$.

The PGP groups were determined in the hemolysate by starch gel electrophoresis following the method of BARKER and HOPKINSON (1979) modified by MARTIN et al. (1981). With the exception of PGP 3 all types were included in the investigation.

For the Bf determination the sera were separated immediately after the blood samples were drawn or received and stored as follows:

1. at room temperature.
2. at $4^{o}C$.
3. at $-25^{o}C$.

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The Bf groups were determined by immunofixation after electrophoresis on cellogel foils according to the method of MARTIN and ZIEGLER (1981). All Bf types we found were included in this investigation.
In addition the Bf groups from blood samples drawn for alcohol tests were investigated after storage at 4°C for 2-13 months and compared to the Bf types determined in freshly drawn blood samples of the same persons.
The following results were obtained:

1. In those hemolysates that were prepared at once or after a 3-days storage at 4°C the PGP patterns could be determined without any alteration for at least two months. During the third month this was not possible any more since the heterocygous types showed vapid and strongly weakened spots.
2. With the blood samples stored at room temperature and later at 4°C safe PGP determination was possible up to the eleventh day.Afterwards the PGP types could not be determined with the heterocygous types any more in spite of storage of the hemolysates at -18°C. With homocygous types correct determination was possible in some cases. The results agree in principle with those described by BRINK et al.(1981).
3. With the hemolysates prepared at once and stored at -40°C determination was unaltered after two years and the PGP typing was as correct as right after the samples were drawn.
4. The Bf types of serum samples stored at room temperature were determinable exactly only for two days. During the next three days some patterns could still be identified, others could not. Bf FS for instance showed only a washed-out area. After the fifth day a correct determination was not possible any more.
5. With the sera stored at 4°C an exact determination of Bf of all types was possible up to the tenth day, but from then on during the next two days the determinability decreased rapidly, so that from the thirteenth day no safe results could be obtained any more.
6. With the sera stored at -25°C completely exact determination of Bf groups could be obtained for at least ten months.
7. Amazingly the Bf typing of 27 partly very old, hemolytic and by no means well stored blood samples from alcohol tests showed results that were identical with those obtained right after the blood was drawn with 22 out of 27. This means a rate of success of 81,5% (with confidence

limits between 61% and 93%).

The results allow the following conclusions concerning the best storage conditions for a successful redetermination of PGP and Bf types in blood samples, hemolysates and sera:

1. PGP groups can be determined exactly for at least two years with hemolysates that are prepared immediately after the blood is drawn or the sample is sent and stored at -40°C.

Differing storage conditions shorten the redetermination interval in direct relation to the storage temperature and the lapse of time until preparation of the hemolysates.

2. Bf groups can be determined exactly for at least ten months in sera that are decanted right after the drawing of the blood or the receipt of the samples and stored at -25°C.

Other storage conditions shorten the time when an exact determination is possible.

3. Surprisingly in sera of hemolytic or otherwise altered blood samples an exact determination of Bf groups is obviously possible in many cases when the blood samples are stored as soon as possible after the drawing (1-5 days) at 4°C for a longer time.

As the determination of blood samples for alcohol tests that were 2 - 13 months old showed the rate of success, 81,5%,was unexpectedly high.

Summary

Phosphoglycolate phosphatase (PGP) and properdin factor B (Bf) groups can be determined or redetermined with success in stored blood samples, hemolysates and sera when optimal storage conditions exist. In freshly prepared hemolysates stored at -40°C the PGP groups can be determined for at least 2 years. In freshly separated sera stored at -25°C the Bf groups are determinable for at least 10 months. The more unfavourable the storage conditions - storage at 4°C or at room temperature and storage of blood samples instead of hemolysates or sera - the shorter the period of determinability. Surprisingly in blood samples drawn for alcohol tests and stored at 4°C for 2 - 13 months the Bf groups were determinable in about 80%.

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