METHODS

A RAPID METHOD OF SCANNING ELECTROPHOROGRAMS

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Paper electrophoresis for separating serum proteins has become widely used. It is very time-consuming to the electrophorograms by eluting the dye from the cut-up paper strip. Densitometers have been described for rapid electrophorogram scanning, but they are at present difficult to obtain.

We used an EKP-4m electrocardiograph, modified as little as possible so it could still be used for its main job, to scan the electrophorograms. The additional devices could be partially produced from apparatus to hand.

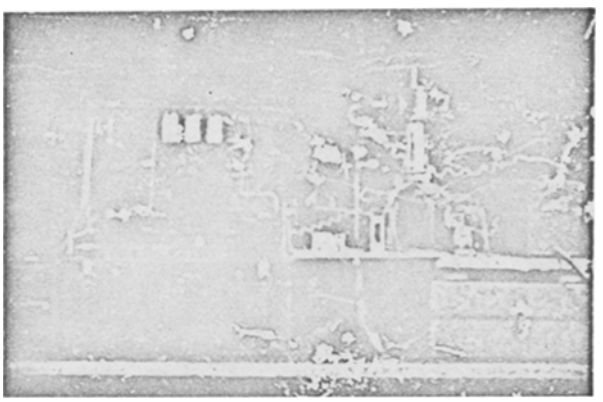

Fig. 1. A general view of the EKP-4m electrocardiograph plus the additional devices.
1) Photomultiplier or photocell; 2) light source; 3) control arc; 4) spool carrier with the electrophorogram strip mounted on it; 5) tension roller; 6) drive roller (drive from a synchronous motor plus reduction gear); 7) plate supply; 8) stepdown transformer; 9) stand.

Flynn and Mayo's [2] and Gurvich's [1] methods were used for serum protein electrophoresis. The strips were prepared from filter paper.

An advantage of this method is that it is relatively simple. No sensitive reflecting galvanometers (electrometers) requiring special locations are used, so the apparatus can be rapidly moved about and set up.

Figure 1 gives a general view of the electrocardiograph plus additional devices.

When the additional devices are being set up care must be taken not to use a beam over 0.5 mm in width and length 3-4 mm less than that of the strip from the light-source.

The photocell current is proportional to the light intensity. When the strip is drawn between the light source and photocell a light beam of variable intensity is produced. The photocell current changes are amplified and transmitted to the electrocardiograph galvanometer and recorded on the film.

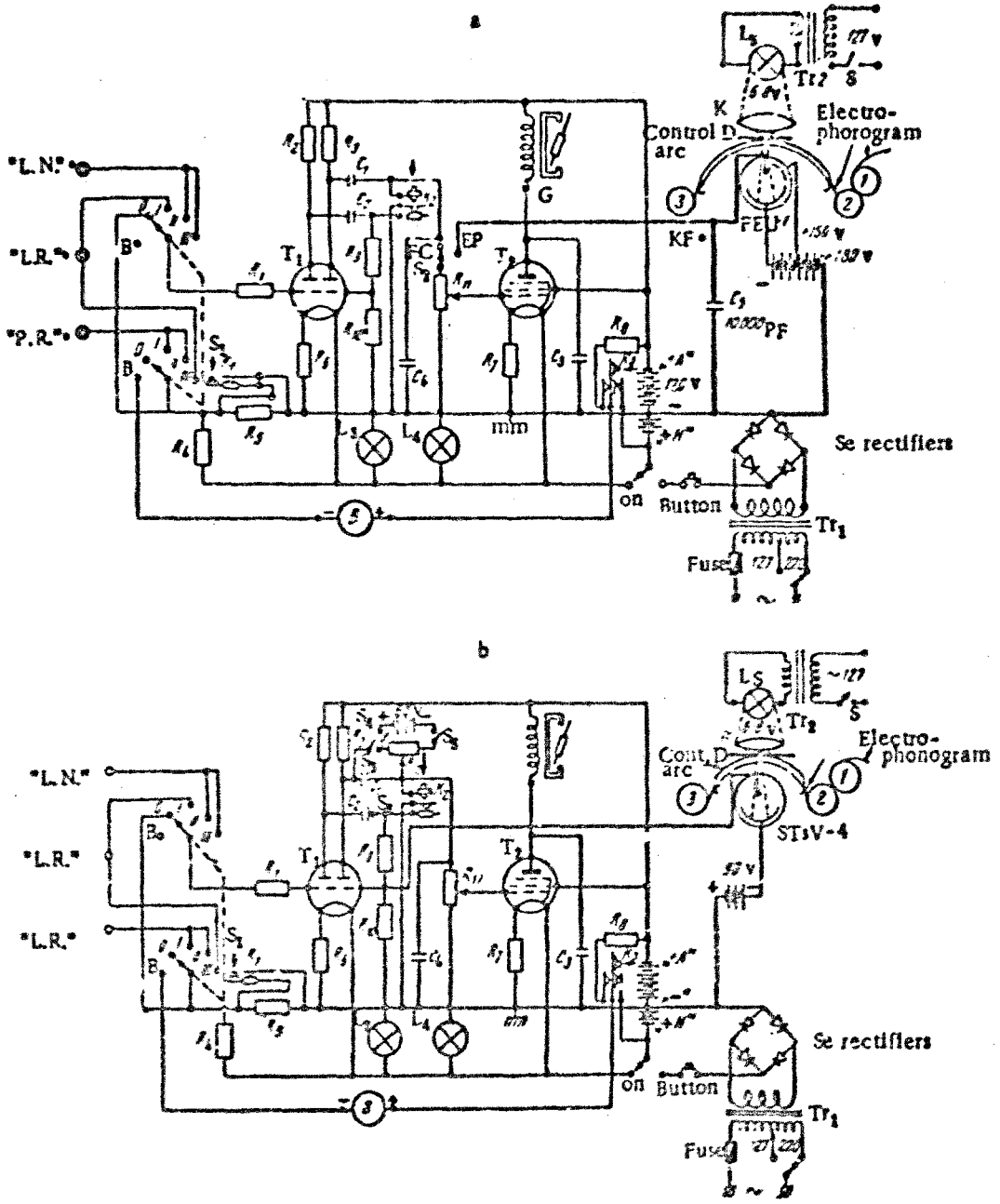

Fig. 2. Basic circuit of the EKP-4m adapted for scanning electrophorograms: First and second variants (a and b respectively). 1) Drive roller; 2) tension roller; 3) spool carrier with the strip mounted on it; L_5 light source, K condenser lens, D diaphragm. The other parts are explained in the text.

The electrocardiograph had all the units required for recording the photocell current. We tested two variants, one with a FEU-1 photomultiplier and the other with a STsV-4 photocell.

• Transliterated from Russian. No explanation given in text.

In the first the Switch S_2 (Fig. 2a) was fitted to the top panel of the EKP-4m; this enabled one to connect the top end of R_{11} either as normally in the EKP-4m ... to the positive side on the FEU-1. In the latter case R_{11} is the photocell load, which supplies the voltage to be amplified to the grid of the amplifier tube T_2 (SO-244). A 10,000 μF capacitor was connected across R_{11} to eliminate 50 cycle line-frequency interference and pulsations due to the low thermal inertia of the lamp filament.

The plate battery voltage of the EKP-4m had to be raised to 120 v.

In the second variant two stages of the EKP-4m amplifier had to be used. The positive side of the STsV-4 was connected to the right-hand grid in T_1(SO-243). A BAS-60 battery was installed in the EKP-4m with a 0.3 MΩ variable resistor (R_{12}) together with a multibank switch to cut in this circuit, which is more complex than that of the first variant (Fig. 2b). This multibank switch does the following operations: cuts out C_2 (S_2), cuts in the 0.3 MΩ variable resistance (R_{12}) in parallel with C_1(S_3), and connects the BAS-60 battery to R_{12} (S_4 and S_5). The check switch must be set at 0 and the gain switch on the 10th gain position, i. e., hard right.

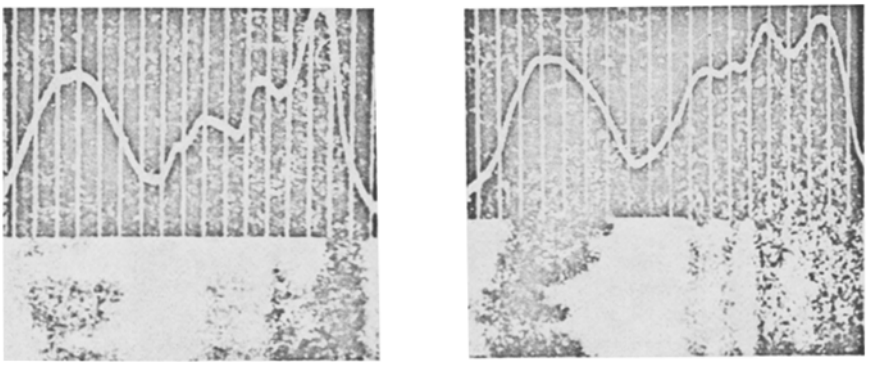

Fig. 3. Electrophorograms from serum proteins of two rabbits with different immunities.
The curves taken on the EKP-4m are shown at the top.

When electrophorograms are being scanned the end of one is fixed to the beginning of the next. A strip comprised of 15-20 such electrophorograms is then wound into a spool (Fig. 1, 4). The front end of the strip (which is an extra piece of paper, and not the first electrophorogram) is inserted into the control arc and gripped between the drive (6) and tension (5) rollers; the apparatus and light source (2) are then switched on.

When the first variant is used the indicator spot on the EKP-4m should be adjusted to the lower part of its range (using the corrector) with a white section of the first electrophorogram under the light source; then the most deeply stained part is placed under the source, and the indicator spot should pass to the other extreme position. The indicator spot can be set to this position by adjusting the light source intensity (rheostat, transformer). This operation should be repeated twice.

Having thus set the white and blue levels the recording will occupy the full width of the film.

When the second variant is used the indicator spot should be set at the center of its range and the multibank switch (S_2, S_3, S_4 and S_5) set to record electrophorograms.

Since the indicator spot does then not appear in its former place it should be returned to the proper position by adjusting R_{12}. The white level will then be at the top of the scale and the blue at the bottom. The white and blue levels are then set.

Then, in both variants, the film-drive mechanism in the electrocardiograph is switched on as well as the synchronous motor (6) which drives the electrophorograms. When all the electrophorograms have passed before the photomultiplier (1) the electrocardiograph and synchronous motor are switched off and the film removed from the cassette and developed.

With the developed image mounted on transparent squared paper the area corresponding to each protein fraction is determined and the percentage relations calculated (see Fig. 3). The total amount of protein being known (from refractometry) the absolute protein fraction contents in the serum can be determined when the time variations of the protein fractions are being studied in a given case.

Chromatograms etc. can be scanned in the same way.

Thus this method amounts to counting the areas of the protein fractions, since the time taken to get the graphic record is practically the same whether one or many electrophorograms are made up into the strip.

The results obtained with the electrocardiograph were checked by eluting the dye and photometering the solutions on an FEK-M, the following results (Table) being obtained.

Method	Type of Measur.	Albumen	α-Globulin	β-Globulin	γ-Globulin
	Area	83	39	33	45
	%	41.5	19.5	16.5	22.5
Elution	Optical density	0.366	0.152	0.155	0.224
	%	40.8	17.0	17.3	24.9
EKP-4m	Area	322	146	108	140
	%	45	20.4	15.1	19.5
Elution	Optical density	0.4	0.18	0.109	0.141
	%	47.8	21.7	13.3	17.2

The data show that the maximum discrepancy was 2.5-2.8%, and we cannot for certain say which method was responsible for these small differences.

Using a PS-381 exit collimator we established the concentration limit beyond which the linear ratio between the dye adsorbed by the protein and light absorption was not obeyed. The linear ratio held up to 2 mg protein per cm^2 of paper. So we used lesser amounts (less than 0.5 mg per cm^2), so there was no danger of the Beer-Lambert law not being obeyed. But the method can also be used with large amounts of protein. It was shown that the extinction and protein concentration were linearly related up to 128 mg/cm^2. In this case the record should be projected onto semilog paper using a magnifier.

With our method the relation between the protein fractions can be determined quantitatively within 5-6 hours of taking the blood for a large series of serum specimens, since only three hours is required to get a good separation of the protein fractions on the paper under our conditions.

Since electrocardiographs are widely used in most medical and scientific institutions this method could find extensive application in clinical practice.

SUMMARY

Two methods of preparing electrophorograms with the electrocardiograph EKP-4m are described. They depend on the type of apparatus at hand, the photomultiplier FEU-1, or the photocell – STsV-4. Additional devices can be mounted from common laboratory equipment. Results are comparable with electrophorograms prepared by other methods, but the rapidity of preparing whole series of electrophorograms with unbroken graphic reproduction makes this method preferable. If protein concentrations of more than 2 mg/cm^2 are used the record should be projected with a photomagnifier on semilog paper.

LITERATURE CITED

[1] A. E. Gurvich. Lab.delo, 3, 1935.

[2] F. Flynn and P. de Mayo, Lancet, 1951, No. 2.