The Analysis of the Wrappings of Mummy 1770

by

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Introduction

The unwrapping of an Ancient Egyptian mummy is a relatively infrequent event and the opportunities for scientific investigation of such remains have, in consequence, been few; previous reports on the composition of materials used in the mummification process have often been based on methods of analysis of a relatively insensitive or non-specific nature.[12]

The present investigation had three principal objectives: first, to describe and identify both microscopically and macroscopically the nature of the material of the bandages; second, to isolate and characterize the materials which may have been applied to the bandages and third, to compare the results of this investigation with those of previous workers. An empirical approach to the investigation was adopted, influenced partly by previous reports and partly by assumption of the availability of certain plant, animal and mineral products to the Ancient Egyptians.

Bandages used to wrap mummies were almost invariably prepared from the fibres of the flax plant (*Linum usitatissimum* L.).[2,12] The use of gum, rather than glue, to secure the bandages around the corpse was mentioned by Herodotus.[9] The source of such gum would probably have been *Acacia* species, possibly *A. senegal*, indigenous to the Upper Nile region. Glue, the residue obtained by the extraction of animal products such as skin, bone and cartilage with boiling water and evaporation of the solvent, was known in Ancient Egypt and used for a variety of purposes,[12] although its use as a bandage adhesive does not appear to have been documented.

The use of waxes by the Ancient Egyptians seems to have been confined to beeswax which has been found covering the ears, eyes, nose, mouth and embalming incision of a mummy.[12] Plant resins and gum resins, such as colophony, storax, mastiche, myrrh or olibanum were probably available to the embalmer and for application to wrappings.[12]

There has been some debate as to whether bitumen from the Dead Sea was used in mummification. Early references to this include those by Diodorus[3] and Strabo.[18] Abraham[1] quotes a reference to the use of bitumen both in the corpse cavities and to coat wrappings from about 500 B.C. to about 40 B.C. Spielman,[20] using ultra violet radiation and spectrographic analysis of the ash concluded that a series of black mummy materials which he examined occupied positions 'between undoubted bitumen and undoubted resins'. The presence of bitumen in a mummy of the Persian period was claimed by Zaki and Iskander[28] on the basis of detection of vanadium, molybdenum and nickel by spectrographic analysis, these elements being characteristic of bitumen.

The present investigation afforded an opportunity of evaluating the applicability of various current methods of analysis, particularly chromatography, in the identification of natural products used in mummification.

Experimental and Results

Bandages from four depths of the mummy wrapping (ranging from the outer wrapping — 1770/1 to the inner bandages overlying the skeleton — 1770/4) were examined at each stage of the investigation.

A: Bandage Fabric

(a) *Macroscopical description* The bandages from the outer layer (1770/1) were in the form of folded strips of fabric, paler brown and less brittle than the bandages of parts 2, 3 and 4 which were irregularly-shaped pieces of very dark brown, fragile material.

1. Weave description: fabrics of different weave were found as illustrated (**1**). Small samples with either hemstitched edges or fringing were also included.

2. Weave density: numbers of threads per cm were determined using a linen tester. The values (averages from four determinations) are given in Table 1.

In the absence of selvedges it was not possible to distinguish warp and weft so in each case the lowest number of threads/cm is quoted first.

(b) *Microscopical examination of fibres* Fibres from all four parts of the bandages were examined after solvent extraction and drying (see section C (b)). The fibres were examined in water (after wetting with alcohol) and compared with authentic flax fibres (**2**). The fibres from the bandage were 9–21 μ diameter and those from flax 12–30 μ. It was not possible to measure the length of the fibres because they were broken. The reactions of the bandage fibres with 1 per cent alcoholic phloroglucinol solution and hydrochloric acid, N/50 iodine and sulphuric acid and cuoxam solution indicated that they were of non-lignified cellulose.

B: Surface Deposits on the Bandages

(a) *Fine grey-white deposit* This was present on small areas of some bandages but was difficult to separate from the bandage fibres and therefore analysed *in situ* by x-ray electron microscopy. The results indicated the presence of the elements: Na, Mg, Si, P, S, Cl, K, Ca and Fe with Na, Cl and S predominant.

(b) *Adhesive* Several pieces of outer strip bandage bore patches of adhesive, fragments of which were scraped off or removed from the bandage by soaking in water and examined as follows:

1. Hydrolysis and paper chromatography 0.36 g glue was dissolved in water and diluted to 40 ml. 4 ml H_2SO_4 was added and the solution heated under reflux on a boiling water bath for 3 hr. The excess acid was neutralized using $BaCO_3$ and the solution examined by ascending PC on Whatman no. 1 paper. Solvent system: *n* BuOH/HOAC/H_2O 4:1:5.

Visualization of spots: (i) spray with aniline phthalate reagent (see p. 123) and heat at 105° for 2–5 min. (ii) spray with ninhydrin solution (0.1 g in 70 ml EtOH) and warm with a hot air dryer.

The results indicated that amino acids were present but monosaccharides absent. For comparison, a sample of gelatin was hydrolysed in a similar manner to the above and co-chromatographed with the glue from the bandage, together with solutions of L-proline and L-OH proline. The results are given in Table 2.

2. Amino acid analysis The results of automated amino acid analysis are given in Table 3.

C: Bandage Impregnation

(a) *Bitumen*

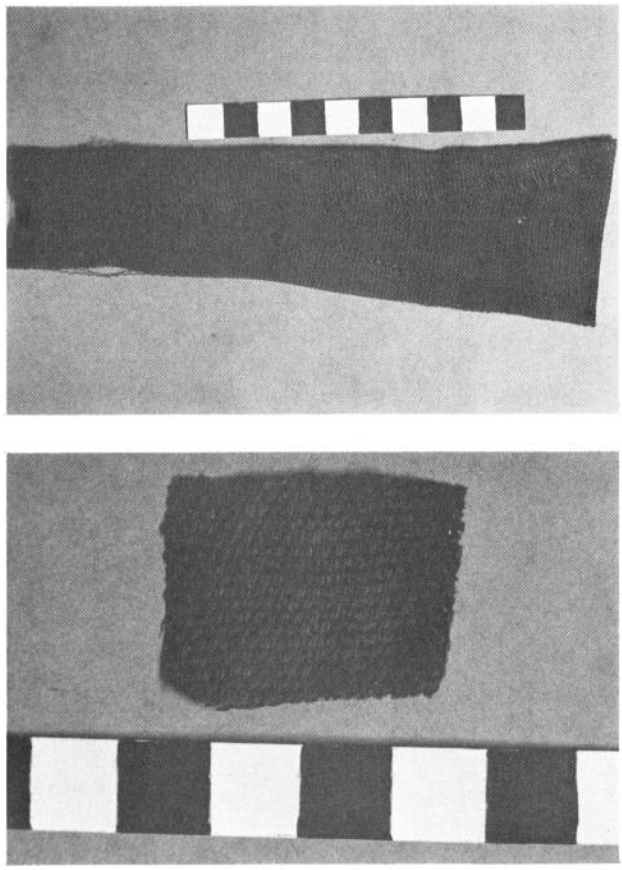
1. Lassaigne Tests were performed on bandage samples from all layers and on samples of Dead Sea bitumen and galbanum. The results are given in Table 4.

2. Tests for Mo, Ni and V were performed using atomic absorption spectroscopy and neutron activation analysis. The results are given in Table 5.

3. Test for Ni was performed on a solution prepared by boiling the ash from approx. 1.8 g bandage with 0.5 ml 10% HCl. A drop of the resulting yellow-green solution was spotted on filter paper, one drop of 1% dimethylglyoxime in EtOH was added and the spot exposed to NH_3 vapour.[25] The resulting precipitate was brown rather than red.

(b) *Solvent extraction of bandages*

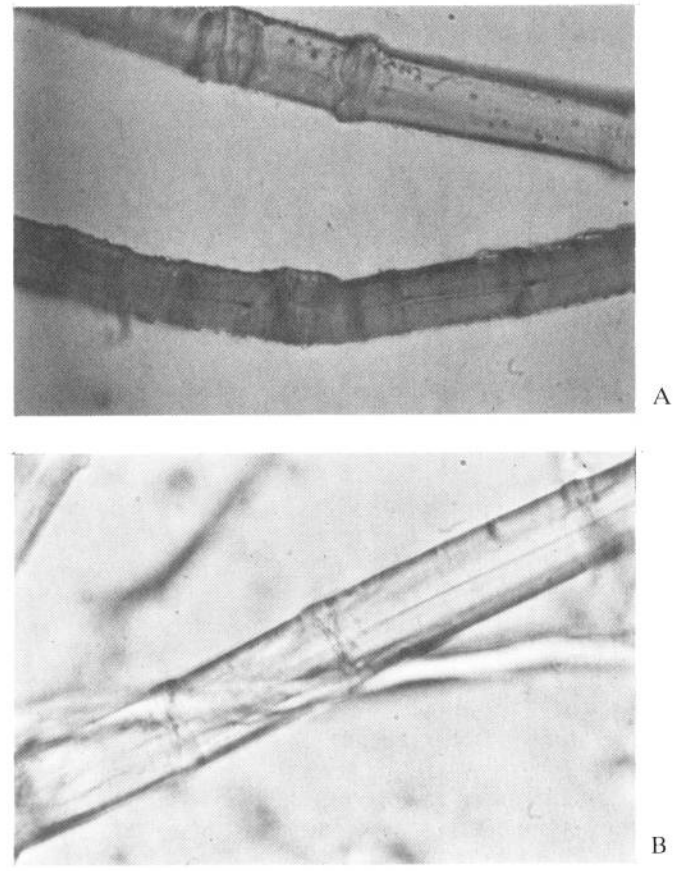
Samples from layers 1–4 of the bandages were extracted with a series of solvents of increasing polarity. Each bandage sample was cut into small pieces, weighed and successively extracted with light petroleum (80°–100°), $CHCl_3$, MeOH and H_2O (approximately 1 L each) in a Soxhlet apparatus. Extraction was continued with each solvent until the extract became colourless (6–18 hr).



A

B

(1) Types of fabric weave (texture) found among the bandages wrapping Mummy 1770 (scale in cms).
(A) Coarse plain weave.
(B) Coarse double weave (double threads in both warp and weft).



A

B

(2) A. Typical fibre from mummy bandage (×630). Features to note include thickened fibre walls, narrow central cavity and 'beat marks'. Compare with authentic linen (flax) fibres, B.

The petroleum, $CHCl_3$ and MeOH extracts were evaporated to dryness under reduced pressure and the residues weighed. The aqueous extracts were concentrated to approx. 300 ml each. The residual bandage was dried to constant weight. The extractives of the bandage samples from each of the four parts are summarized in Table 6.

(c) *Chromatographic examination of residues*

1. Light petroleum extracts

(i) Thin layer chromatography

Stationary phase: silica gel G 0.25 mm, activated at 110°, 30 min.

Mobile phases: A CCl_4[10]
B $C_6H_6/CHCl_3$ 7:3[10]
C *n* hexane/Et_2O/HOAc 90:10:1[21]

Visualization of spots: (a) examine plate in UV light (365 nm); (b) spray with 0.05% aqueous Rhodamine 6G[10] *or* spray with 20% alcoholic phosphomolybdic acid, heat at 105–110° for 5–10 min and examine in daylight.[21]

Reference substances: see Table 7.

The results of the chromatograms are summarized in Table 7.

(ii) Gas Liquid chromatography

Gas chromatograph: Perkin Elmer F11 equipped with F.I.D., carrier gas (N_2) at 30 ml/min and the following stationary phases packed in 2 mm i.d. × 180 cm columns:

A 15% Carbowax 20 M on Chromosorb P (oven temp. 200°);
B 4% SE 30 on Gas Chrom Q (oven temp. 200°);
C 10% OV 17 on Chromosorb P (oven temp. 280°).

Reference substances: see Table 8.

The results of GLC examination are summarized in Table 8 and (**4**).

(iii) Column chromatography: 1 g light petroleum extract from 1770/1 was applied to a column of approx. 100 g silica gel (100–120 mesh) in light petroleum (80°–100°). Elution with 150 ml light petroleum gave the hydrocarbon fraction of the wax (approx. 900 mg) and with $CHCl_3$ (100 ml) the ester fraction (64 mg).

(iv) Preparative GLC:

Gas chromatograph: Varian Aerograph Autoprep 705 equipped with F.I.D., N_2 at 30 ml/min, oven temp. 200° and a 4 mm i.d. × 200 cm column packed with 20% Carbowax 20M on Chromosorb P.

Fractions were collected corresponding to the peaks having the following R_t (system A): 12.0, 17.0, 24.4, 35.0, 50.2 and 71.6. Each fraction was examined by mass spectrometry (70 eV, inlet temperature 200°) and the data (presented in Table 9) indicated a series of *n*-alkanes. Fractions corresponding to the peaks with R_t 17.0, 35.0 and 71.6 were also collected from yellow beeswax by a similar procedure. The MS were closely similar to those from the bandage extracts. A graph of log R_t against carbon number plotted for the six *n*-alkanes isolated from the bandage petroleum extract allowed the prediction of carbon numbers for those not isolated (**3**).

(v) Preparative TLC of the ester fraction from (iii) resulted in 5.1 mg of the ester fraction free from *n*-alkane. The corresponding fraction of yellow beeswax was obtained for comparison, using the same procedure. The MS (conditions as in (iv)) are summarized below:

ester from bandage extract, ions > m/e 200: m/e (% relative abundance) 732 (6), 704 (16), 676 (24), 648 (10), 620 (4), 465 (4), 437 (6), 420 (6), 392 (10), 313 (24), 285 (100), 267 (12), 257 (100), 239 (12);

ester from beeswax: 704 (24), 676 (50), 648 (35), 620 (33), 592 (46), 448 (4), 420 (10), 336 (13), 285 (7), 257 (100), 239 (17).

2: Chloroform extracts

Resins such as those mentioned on page 119 would have their maximum solubility in chloroform so principally the extracts prepared with this solvent were examined for resins but, as shown in (**5**) and Table 10, the petroleum and methanol extracts were also compared with authentic resins.

(i) Thin layer chromatography

Stationary phase: silica gel G 0.25 mm, activated at 110°, 30 min.

Mobile phase: C_6H_6/MeOH 95:5, double development using 'S' chamber.[21]

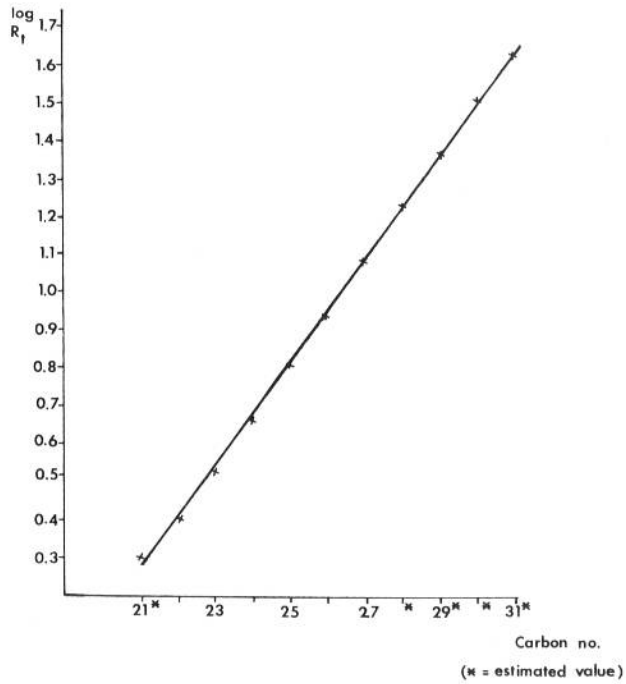
Visualization of spots: (a) examine plate in UV light (365 nm); (b) spray with $SbCl_3$ in $CHCl_3$ followed by heating at 100°C for 10 min.

Reference substances: see Table 10.

(ii) Gas Liquid chromatography

Conditions: Systems A and B as described opposite.

Results: the chromatograms indicated an absence of peaks due to wax hydrocarbons from the $CHCl_3$ and MeOH extracts and no additional information was obtained, other than that from the chromatograms of the light petroleum extracts.



(**3**) Graph of carbon number against (log R_t) (System B) for *n*-alkanes from light petroleum extract of 1770/1 wrappings.

(iii) Isolation of umbelliferone

A saturated $CHCl_3$ solution of the $CHCl_3$ extract of 1770/1 bandages was applied to an alumina column (approx. 7 cm × 0.8 cm) and eluted with approx. 3 ml $CHCl_3$. The eluate was concentrated and subjected to preparative TLC by the system used in (i) above, except that the mobile phase was $C_6H_6/CHCl_3$ 95:5. The blue fluorescent band (UV, 365 nm) was eluted with $CHCl_3$ and the concentrated eluate compared on TLC with umbelliferone and galbanum as follows:

Stationary phase: Systems A and B — silica gel G, 0.25 mm, activated at 110° for 30 min.
System C — cellulose powder 0.3 mm, air dried.

Mobile phases: A $C_6H_6/CHCl_3$ 95:5 (×2, 'S' chamber)
B MeOH/$CHCl_3$ 1:1
C 10% aq. HOAc.[5]

Visualization of spots: examine plate in UV light (365 nm).

Reference solutions: 3% galbanum in $CHCl_3$ umbelliferone in EtOH.

Results: see Table 11.

3. Methanol extracts

(i) Thin layer chromatography: see sections 2 and 4.

4. Aqueous extracts

(i) Spot tests: a series of spot tests was carried out on aqueous extracts of layers 1, 2, 3 and 4, the methods and results of which are given in Table 12.

(ii) TLC of plant acids:

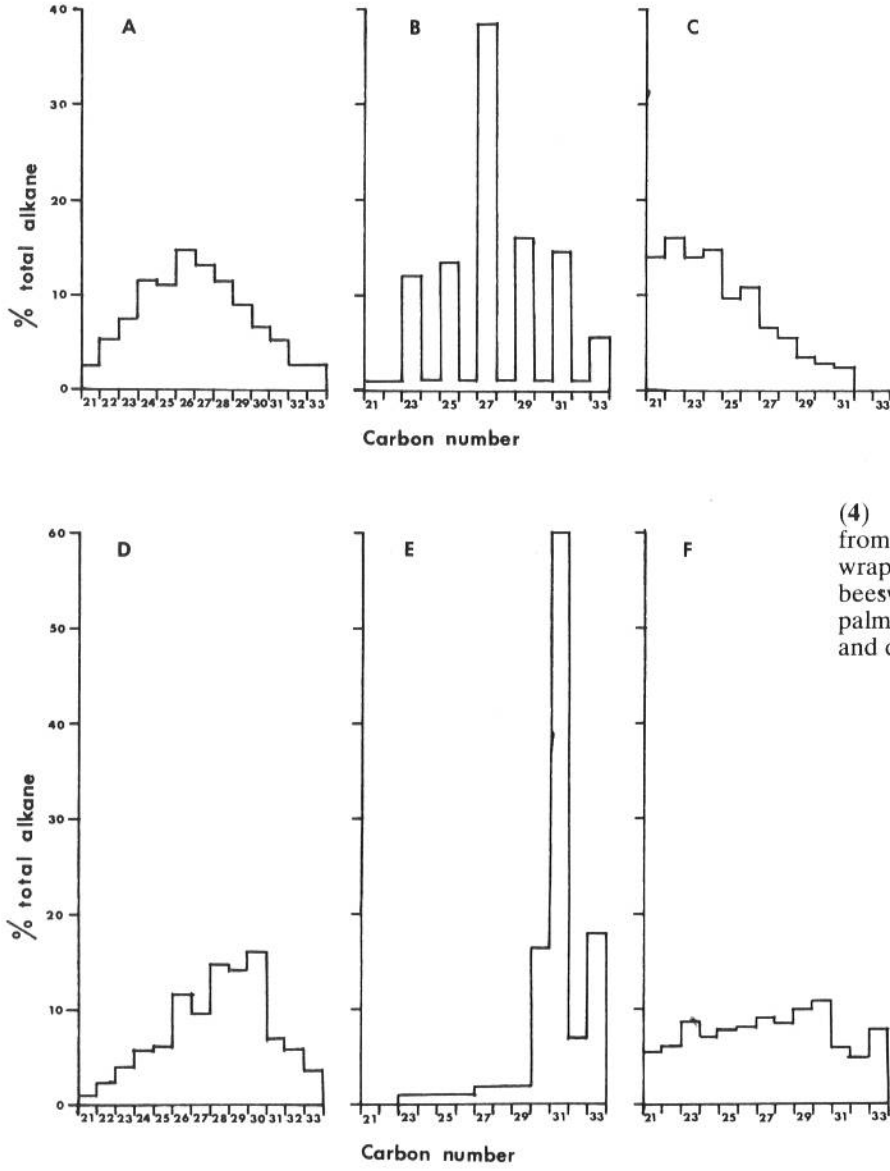
Stationary phase: cellulose powder, 0.3 mm, air dried.

Mobile phases: A *n* BuOH/HCOOH/H_2O 4:1:5 (upper layer)
B *n* PrOH/1M NH_4OH 7:3
C 95% EtOH/NH_4OH/H_2O 8:1:1[5,19]

Visualization of spots: (a) 0.04% bromothymol blue in 0.01 M NaOH; (b) equal volumes of 0.1 M $AgNO_3$ and 0.1 M NH_4OH mixed immediately before spraying.

Reference substances: see Table 13.

Solutions of bandage extracts used: MeOH extracts in MeOH; aqueous extracts after removal of acid-insoluble precipitate by the following method: 10 ml concentrated



(**4**) GLC comparison of *n*-alkanes from petroleum ether extracts of wrappings [1770/1] (A), beeswax (B), hard paraffin (C), palm wax (D), palm leaf wax (E), and date fruit skin wax (F).

H_2O extract was mixed with 1 ml HCl and heated in a boiling water bath under reflux for 20 min., cooled and filtered.

The results of the chromatograms are summarized in Table 13. The MeOH and aqueous extracts from all layers gave similar results so only those from one are quoted.

(iii) Paper chromatography of monosaccharides: Descending chromatography on Whatman No. 1 paper.

Mobile phases: A *n* — BuOH/HOAc/H_2O 4:1:5
B *n* — BuOH/EtOH/H_2O 4:1:5
C PhOH saturated with H_2O.[5]

Visualization of spots: papers sprayed with aniline phthalate reagent (aniline, 1 ml + phthalic acid, 1.66 g in 100 ml *n* BuOH saturated with water) and heated at 105° for 2–5 min.

Reference substances: see Table 14.

To prepare the hydrolysates 1 g powdered gum or gum resin was heated under reflux with 5 ml H_2O and 1 ml HCl for 30 min. in a boiling water bath. The resulting mixture was filtered and washed with $CHCl_3$ (for gum resins) to yield an aqueous solution of gum hydrolysate.

Solutions of extracts used: hydrolysed aqueous extracts as in (ii).

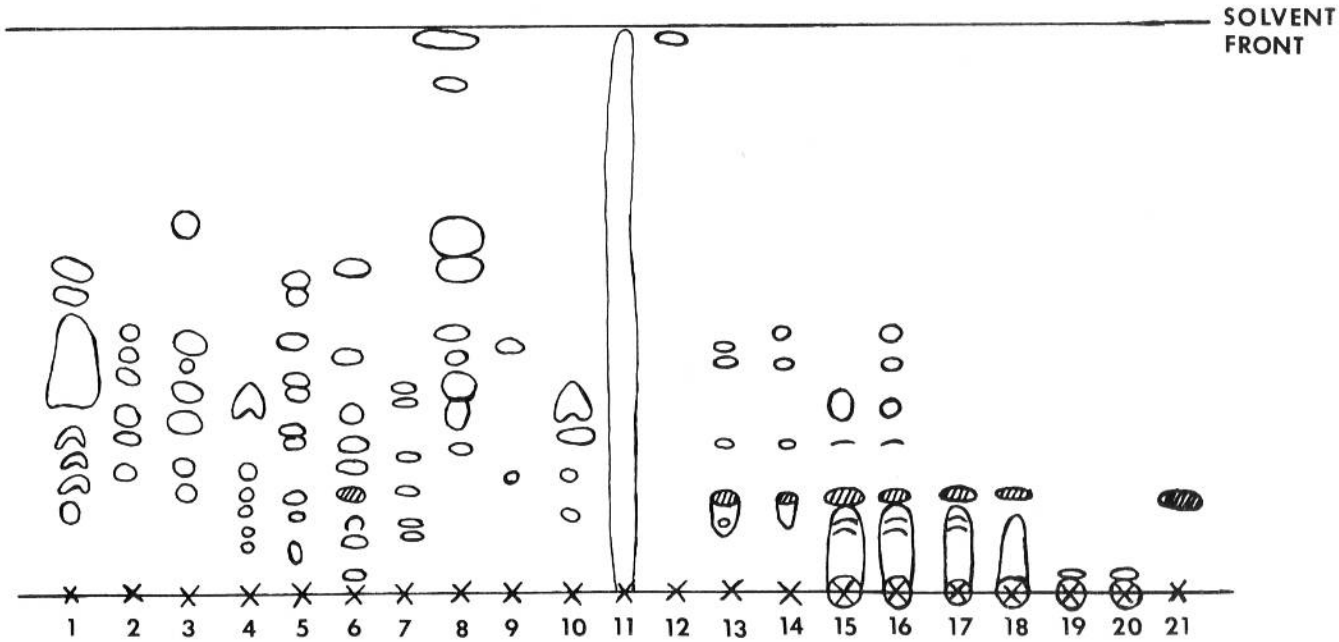
The results of the chromatographs are summarized in Table 14. The aqueous extracts from all layers examined gave similar results so only those from one are quoted.

Discussion

A: Bandage Fibres

An assortment of fabrics (**1**, Table 1) seems to have been used in wrapping the mummy. Near the skeleton quite large sheets of fabric were found, together with smaller pieces with hem-stitched edges or fringes, while strip bandages were used only for the outer layers. This could suggest that the lower layers were not particularly carefully chosen or applied, the uniform folded strips of bandage being reserved for the outer layer.

The only type of fibres which is reliably reported as having been used by the Ancient Egyptians for mummy bandages is flax. Wool, although available was not used in temples or when burying the dead. Of the other natural fibres, cotton, originally from India, and silk (from China) are thought to have been used in fabrics in Roman times or later, but have not yet been found in mummy wrappings; hemp (from an unspecified botanical source), ramie (from *Boehmeria nivea* (L.) Gand., Urticaceae) and kenaf (from *Hibiscus cannabinus* L., Malvaceae) were all indigenous to Egypt but they are coarse fibres and cannot be woven into fine fabrics.[11,12] All these fibres can be distinguished by their microscopical characters.[11,27] The fibres from the bandage fabrics were undoubtedly cellulosic and of plant origin and the diameter (9–21 μ) of the fibres, narrow lumen, pointed apices and cross markings are all strongly indicative of flax (**2**).



(**5**) Thin layer chromatographic comparison of the petroleum ether, chloroform and methanol extracts of the mummy bandages with reference resins.

1. Colophony	2. Chios turpentine	3. Mastiche	4. Sandarac
5. Olibanum	6. Galbanum	7. Ladanum	8. Myrrh
9. Bdellium	10. Storax	11. Dead Sea asphalt	12. Pet. ether 1
13. Pet. ether 2	14. Pet. ether 3	15. $CHCl_3$ 1	16. $CHCl_3$ 2
17. $CHCl_3$ 3	18. MeOH 1	19. MeOH 2	20. MeOH 3
21. Umbelliferone			

B: Surface Deposits

(a) *Grey-white deposit* The identity of the fine greyish-white deposit on parts of the bandage was not conclusively established since there were several elements present. The predominance of Na, K, S and Cl might suggest that it was composed of the chlorides and sulphates of sodium and potassium from a crude mineral source.

(b) *Adhesive* The glue on the surface of some of the outer strip bandages was expected to be a gum of plant origin, e.g. Acacia[9] but hydrolysis and a preliminary paper chromatogram indicated that it was protein rather than polysaccharide. The presence of L-OH proline in the hydrolysate suggested that the gum was a collagen-derived protein since this amino acid is otherwise rare.[13] The results of chromatography and amino acid analysis of the glue hydrolysate (Tables 2 and 3) indicated that the glue was gelatin of very similar amino acid composition to that of the modern product. The presence of gelatin is somewhat surprising in view of the taboo, reported by Herodotus,[9] on the use of animal products in mummification.

C: Bandage Impregnation

(a) *Bitumen* Spielman[20] and Zaki and Iskander[28] have suggested that bitumen (asphalt) from the Dead Sea was used in the preparation of mummy wrappings. Bitumen consists of a colloidal suspension of carbon in a hydrocarbon oil which contains about 6–8% sulphur.[26] As such, it has a rather low solubility in any solvent so tests were performed on the whole bandage rather than extracts. The Dead Sea bitumen used for comparison could be dispersed in light petroleum (80–100°) but the resulting suspension gave no useful thin-layer or gas-liquid chromatograms. According to Spielman the presence of the metals molybdenum, nickel and vanadium, as well as sulphur can be used as a means of identification of bitumen in mummies.

Lassaigne tests on the wrappings of 1770 (Table 4) showed that sulphur was absent from the outer layer of the bandages but present in layers 2, 3 and 4 and in the bitumen used for comparison. Galbanum was included in the tests because subsequent results indicated its presence in the bandage. The identification of nitrogen and halogen in all layers of the bandage is not surprising in view of their presence in galbanum.

The results of the analyses for Mo. Ni and V (Table 5) were positive for Mo and V in both the bandage and Dead Sea bitumen although Ni could not be detected in the bandage by any of the methods used. This may be due to the relative insensitivity of the methods but means that the evidence for the presence of bitumen in the wrappings is incomplete, although the detection of S, Mo and V provides a strong indication of its use in the lower layers.

(b) *Solvent extraction* The yields of extractives to various solvents (Table 6) indicate a marked difference between the outer bandages and those nearer the skeleton; 1770/1 bandages gave a higher petroleum extractive than any of the other layers but contained much less water-soluble material. Beneath the outer layers, the amount of petroleum-soluble matter decreased towards the interior but there was no consistent trend in any of the other values.

(c) *Chromatographic examination of extracts* The results of the examination of the petroleum extractive (Table 7) suggested the presence of beeswax in all four layers of the bandage, with spots corresponding to the *n*-alkane and ester fractions being most prominent.[10] Beeswax consists of 70–80% myricyl palmitate ($C_{15}H_{31}COOC_{30}H_{61}$) together with free cerotic acid ($C_{26}H_{53}COOH$) and various minor components,

Table 1 Weave densities of mummy bandage fabrics

Bandage from part no.	threads/cm	threads/cm
1770/1 (single weave)	8	18
2 (single weave)	8	18
2 (double weave)*	12	18
3 (single weave)	11	32
3 (single weave)	8	23
3 (double weave)*	14	22
4 (single weave)	13	22
4 (single weave)	16	17
4 (double weave)*	14	22

*numbers quoted are of single threads, e.g. 12 single threads = 6 double threads

Table 2 Results of comparison of hydrolysis products of glue from bandages with those of gelatin

hRf	Colour with ninhydrin	Presence (√) or absence (—) in solutions of:			
		L-proline	L–OH proline	glue hydrolysate	gelatin hydrolysate
61	purple	—	—	√	√
46	purple	—	—	√	√
38	yellow	—	—	√	√
31	yellow	√	—	√	√
27	purple	—	—	√	√
23	purple	—	—	√	√
20	yellow	—	√	√	√
14	purple	—	—	√	√

TABLE 3 Results of Amino Acid analysis of glue from bandages and gelatin

Amino Acid	Residues per 1000 total AA residues	
	Bandage Glue	Gelatin
OH-Pro	99.05	101.00
Asp	46.61	47.86
Thr	17.78	19.21
Ser	36.76	35.79
Glu	81.71	80.48
Pro	127.92	125.77
Gly	288.48	305.80
Al	115.30	106.21
Cyst	0.0	0.0
Val	22.76	19.94
Meth	7.20	6.41
Ileu	11.02	10.69
Leu	29.75	26.75
Tyr	3.66	2.46
Phe	14.95	12.88
OH-Lys	11.29	13.08
Lys	28.28	27.86
Hist	8.92	8.10
Arg	48.55	49.70

TABLE 4 Results of Lassaigne tests on Bitumen, Galbanum and the mummy bandages

Material	Presence (√) or absence (—) of		
	S	N	Halogen
Dead Sea Bitumen	√√	?	(√)
Galbanum	—	√	(√)
Bandage 1770/1	—	√√	(√)
2	√	√	(√)
3	√	√	√
4	√	√	√

TABLE 5 Results of tests for Mo, Ni and V in Bitumen, Galbanum and the mummy bandages

Element	Content of Element (ppm) in:		
	Mummy bandage (1770/3)*	Dead Sea Bitumen	Galbanum
Mo	93.8 (AAS)†	219.3 (AAS)	<level of detection (AAS)
Ni	—‡	251.0 (AAS)	<level of detection (AAS)
V	11 ± 1 (NAA)	463.0 (AAS)	<level of detection (AAS)

* Additional elements present in mummy bandage (NAA): Mn 41±3 ppm; Al 1800±40 ppm; Na 0.33±0.04%; Cl 0.45±0.06%.
† Techniques used: AAS = Atomic Absorption Spectroscopy;
NAA = Neutron Activation Analysis.
‡ i.e., below level of detectability.

TABLE 6 Extractives of bandages

Part	Weight bandage (g)	% Extraction*				
		Light Petroleum 80°–100°	$CHCl_3$	MeOH	H_2O	Total
1770/1	87.745	8.8	4.7	3.4	0.3	17.2
2	101.415	2.8	9.7	15.0	21.6	49.0
3	28.165	1.9	8.6	7.0	30.0	48.0
4	30.040	0.8	4.5	3.1	30.5	39.0

* The light petroleum extracts were coloured as follows: from 1770/1 — pale yellow, layer 2 — orange-yellow, layers 3 and 4 — deep orange. All other extracts were very dark brown in colour.

principally a series of *n*-alkanes including nonacosane (C_{29}) and hentriacontane (D_{31}).[22,23]

In 1770/1 the spot corresponding to the hydrocarbon fraction of the wax was larger relative to the ester than in authentic beeswax, possibly suggesting either the presence of hydrocarbon from another source, or decomposition of the ester on storage. The chromatograms from the extracts of layers 2, 3 and 4 showed a yellow fluorescent streak and the extracts themselves have a bright orange colour, suggesting the presence of resin or other pigmented material in the lower layers. A mixture prepared by melting together galbanum and beeswax gave a similar colour and yellow streak on TLC, although the baseline spot fluoresced blue rather than yellow. The results of GLC of the petroleum extracts of 1770/2, 3 and 4 also suggest that a mixture of beeswax and galbanum may be present (Table 8).

MS of the ester fraction of the wax from the extract indicated clearly the presence of myricyl palmitate ($CH_3(CH_2)_{14}\underset{\underset{O}{\|}}{C}–O(CH_2)_{29}CH_3$) with M^+ at m/e 676 and principal fragment ions at m/e 257 ($[C_{16}H_{33}O_2]^+$), 239 ($[CH_3(CH_2)_{14}C{=}O]^+$), 420 ($[C_{30}H_{60}]^+$) and 465 ($[CH_3(CH_2)_{29}–O–C{=}O]^+$). The ions at m/e 257 and 420 are considered to be derived by fission of the alkyl-oxygen bond with rearrangement of 2H from the alkyl group in the former and loss of H from the latter ([17] and references therein). In addition to the myricyl palmitate, there were ions suggesting the presence of higher homologues at M^+ m/e 704 and m/e 732.

The former is most probably due to a small amount of myricyl stearate since the fragments at m/e 285 and 267 would form from a stearate ester, while ions at m/e 448 and 293 which would be expected from a C_{32} alcohol palmitate are absent. The ion m/e 732 probably represents a trace amount of myricyl arachidate ($CH_3(CH_2)_{18}–\underset{\underset{O}{\|}}{C}–O–(CH_2)_{29}–CH_3$) since the corresponding acid fragment at m/e 313 is observed in the spectrum. The esters of the wax are of too high molecular weight to be eluted from GLC columns under the conditions used. The peaks observed (Table 8) were shown by MS to be due to a series of *n*-alkanes of carbon number C_{21}–C_{33} (**3**). However, the quantitative differences in peak area observed for beeswax, in which the odd carbon number *n*-alkanes predominate, were not observed in the bandage extract. This suggests the presence of hydrocarbon from another source. In plant and insect waxes, odd numbered carbon *n*-alkanes generally predominate probably due to their biogenesis by the decarboxylation of even carbon

TABLE 7 Results of TLC comparison of petroleum ether extracts of mummy bandages with beeswax, beeswax and galbanum, and hard paraffin

hRf	Class of wax constituent[10]	Presence (√) or absence (—) of spot in						
		Beeswax	Beeswax + galbanum	Hard paraffin	Petroleum ether extracts of layers			
					1	2	3	4
System A								
77	*n*-alkanes	√	√	√	√	√	√	?streak
39	*n*-alkyl esters	√	√	—	(√)	√	√	√
11		√	√	—	—	—	√	√
6	*n*-secondary alcohols?	—	√	—	—	(√)	√	√
0		√	√*	—	(√)	√†	√†	√†
System B								
80	*n*-alkanes	√	√	√	√	√	√	√
73	*n*-alkyl esters	√	√	—	√	√	√	√
52	*n*-secondary alcohols?	(√)	(√)	—	—	—	(√)	(√)
40		(√)	(√)	—	—	(√)	(√)	(√)
20	*n* 1° alcohol	√	√	—	—	√	√	√
15		√	√	—	—	√	√	√
4	*n*-fatty acid	√	√	—	√	√	√	√
0		√	√*	—	√	√†	√†	√†
System C								
75		√	√	√	√	√	√	
62		√	√	—	—	√	√	
51		√	√	—	—	√	√	
39		√	√	—	—	√	√	STREAK
24		√	√	—	—	√	√	
13		√	√	—	—	√	√	
6		√	√	—	√	—	√	
0		√	√*	—	√	√†	√†	†

Footnotes:

* baseline spot fluoresced blue in UV (365 nm), with a yellow fluorescent streak extending from the baseline up the plate.

† yellow UV-fluorescent baseline spot with streak up the plate.

number fatty acids.[4] However, this is not universal and where alkanes form only a minor constituent of plant leaf waxes, as in the genera *Pinus* and *Eucalyptus*, alternation between odd and even carbon numbers through the homologous series is almost indiscernible.[6,7]

The leaf wax of *Copernicia ceriferae* Palmae (Carnuaba wax), shows such a non-alternating pattern as does the stem wax of *Ceroxylon spp*. Palmae ('Palm wax' of commerce) see (**4**). As both these plants are indigenous to South America, it is unlikely that their waxes would be available to the Ancient Egyptians. The waxes of two other members of the Palmae, *Phoenix reclinata* and *P. canariensis* do not show this anomalous pattern[8] but waxes from the leaves and fruits of *P. dactylifera* (the Date palm), a plant which was used by the Egyptians to produce wine,[2] were examined. Unlike the leaf wax, the fruit wax from this species did show an anomalous *n*-alkane pattern (**4**) but the small amounts of wax which can be extracted suggest that it would be an unlikely material for use in mummification and wrapping procedures. Further, it is reported[24] that the major monoesters of the palm waxes ouricury and carnauba are of

TABLE 8 Results of GLC comparison of petroleum ether extracts of mummy bandage with beeswax, beeswax and galbanum, hard paraffin and other reference waxes

n-alkane carbon no.	21	22	23	24	25	26	27	28	29	30	31	32	33
System A													
Rt. (min)	8.4	12.0	17.0	24.4	35.0	50.2	71.6	98.0	—	—	—	—	—
Beeswax	√	(√)	√	(√)	√	(√)	√	—	—	—	—	—	—
1770/1	√	√	√	√	√	√	√	√	—	—	—	—	—
2*	√	√	√	√	√	√	√	√	—	—	—	—	—
3*	√	√	√	√	√	√	√	√	—	—	—	—	—
4*	√	√	√	√	√	√	√	√	—	—	—	—	—
System B													
Rt.	2.0	2.6	3.4	4.6	6.4	8.6	12.2	17.0	23.4	32.2	43.0		
Beeswax	√	(√)	√	(√)	√	(√)	√	(√)	√	—	√	—	—
1770/1	√	√	√	√	√	√	√	√	√	√	√	—	—
2	√	√	√	√	√	√	√	√	(√)	(√)	(√)	—	—
3	(√)	(√)	(√)	(√)	(√)	(√)	(√)	(√)	—	—	—	—	—
4	(√)	(√)	(√)	(√)	—	—	—	—	—	—	—	—	—
System C													
Rt.	3.4	4.4	5.4	6.8	8.6	10.8	13.7	17.4	22.0	28.4	35.4	45.4	57.4
Beeswax	(√)	(√)	√ (12)†	(√)	√ (13.4)	(√)	√ (38.2)	(√)	√ (16)	(√)	√ (14.2)	(√)	√ (5.9)
Hard paraffin	√ (14)	√ (16.1)	√ (14)	√ (14.7)	√ (9.6)	√ (10.5)	√ (6.4)	√ (5.5)	√ (3.3)	√ (2.9)	√ (2.35)	—	—
'Palm' wax	√ (0.7)	√ (2)	√ (3.9)	√ (5.5)	√ (5.9)	√ (11.5)	√ (9.7)	√ (14.9)	√ (14.2)	√ (15.8)	√ (7.0)	√ (5.9)	√ (3.0)
Date palm leaf wax	—	—	(√)	(√)	(√)	(√)	(√)	(√)	(√)	√ (16.0)	√ (60)	√ (6.4)	√ (17.5)
Date fruit skin wax	√ (5.8)	√ (6.0)	√ (8.3)	√ (7.2)	√ (7.7)	√ (8.0)	√ (9.2)	√ (8.4)	√ (10)	√ (10.6)	√ (5.8)	√ (5.0)	√ (8.0)
1770/1	√ (2.4)	√ (5.0)	√ (7.6)	√ (11.4)	√ (11.1)	√ (14.4)	√ (13.1)	√ (10.9)	√ (8.3)	√ (6.1)	√ (4.8)	√ (2.4)	√ (2.4)
2	√	√	√	√	√	√	√	√	√	√	√	√	√
3	√	√	√	√	√	√	√	√	√	√	√	√	√
4	√	√	√	√	√	√	√	√	√	√	√	√	√

Footnotes:
√ = present; (√) = present (small peak)
* If beeswax is melted with galbanum and a petroleum ether extract subjected to GLC (System A), additional peaks are observed with the following Rt (min):
1.4, 1.8, 2.2, 3.0, 4.6, 5.0, 6.0, 9.6, 13.6, 14.6, 19.6. These peaks were also identified in chromatograms of the petroleum extracts of 1770/2, 3 and 4. The presence of additional peaks in the chromatograms of beeswax + galbanum and of extracts of 1770/2, 3 and 4 caused poor chromatograms in systems B and C. Wool fat, Dead Sea bitumen and olive oil produced no useful chromatograms under the conditions used.
† numbers in brackets indicate % of total *n*-alkane (area measurement).

TABLE 9 Mass spectral data on *n*-alkanes from a light petroleum extract of bandage

R_t (min) (System A)	M^+ (m/e)*	$C_n H_{(2n+2)}$ n =
12.0	310	22
17.0	324	23
24.4	338	24
35.0	352	25
50.2	366	26
71.6	380	27

* In each case the MS indicated an $(M-15)^+$ ion and ions representing successive removals of 14 m.u., characteristic of an unbranched saturated alkane.

TABLE 10 Results of the TLC comparison of the petroleum ether, chloroform and methanol extracts with reference resins

		hRf and colours of spot
Colophony		58, 54, 43, 29, 24, 20, 14
Chios Turpentine		46, 42, 31, 27, 21
Mastiche		66, 45, 41, 36, 30, 23, 18
Sandarac		35P, 22, 17, 14, 11, 8
Olibanum		56, 53, 45, 38, 35, 29, 26, 16, 13, 7
Galbanum		58, 42, 32GR, 26P, 22.5P, (17), 12P, 9
Ladanum		35P, 34GR, 24P, 18GR, 12P, 10
Myrrh		99MB, 91M, 63MB, 57MB, 46, 42, 37M, 32, 25
Bdellium		44, 20
Storax		34, 28, 21, 13
Dead Sea Asphalt		Black streak
1770 Pet. ether extract	(1)	99*
	(2)	43.5, 40.5, 26.5, (17), 12
	(3)	46, 40, 26, (17)
1770 $CHCl_3$ extract	(1)	33GR, 26, (17), 13, 11
	(2)	47, 41, 31.5GR, 26, (17), 13, 11
	(3)	(17), 13, 11
1770 MeOH extract	(1)	(17)
	(2)	3
	(3)	3

Key:
( ) = bright blue fluorescence; P = purple, B = black, GR = green, M = mauve. All other spots grey.
umbelliferone hRf = 17.
* Wax *n*-alkanes, rhodamine positive.

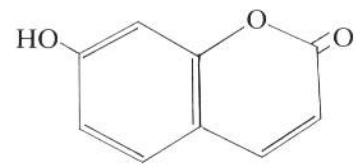
TABLE 11 Identification of Umbelliferone

System	hRf	Presence (√) or absence (—) of spot in solutions of		
		blue fluorescent compound separated from chloroform extract of 1770/1	Galbanum	Umbelliferone
A	14	√	√	√
B	63	√	√	√
C	64	√	√	√

chain length C_{54}–C_{60}, in contrast to those of beeswax in the C_{46}–C_{48} range. The esters in the bandage extract were of similar chain length to those in beeswax and too low for palm wax.

Mineral waxes, which exhibit non-alternating patterns of *n*-alkanes (see **4**, hard paraffin) could have been used. The natural petroleum wax ozokerite, obtained from the shores of the Dead Sea[16] was known to the Ancient Egyptians, beads of this material having been found in some of their graves.[12] Recent GLC analysis shows that ozokerite from the shores of the Dead Sea contains 90% of its *n*-paraffins in the C_{36}–C_{45} range with a maximum at C_{38}.[16] However, in samples from other locations throughout the world, the ranges are lower,[14] therefore it is possible that ozokerite could have been mixed with the beeswax which was applied to the bandages. Other possibilities are that a form of beeswax was used which had a different *n*-alkane content or that paraffin wax was applied to the mummy in modern times.

TLC comparison of the petroleum ether, chloroform and methanol extracts of the wrappings with chloroform solutions of the resins and gum resins previously reported to have been available to the Ancient Egyptians[2,12] failed to give an immediate positive identification of the resin or resins which had been used to impregnate the bandages of 1770 (**5**). This is not entirely unexpected as the volatile compounds present in these products would be lost and others oxidized on storage. However, a blue fluorescent compound (hRf 17, Table 10) present in the petroleum ether and chloroform extracts of the wrappings was also present in the extract of galbanum but not in those of any other resin or gum resin examined. The blue fluorescent component of galbanum is a hydroxy-coumarin, umbelliferone (I).[23]



I

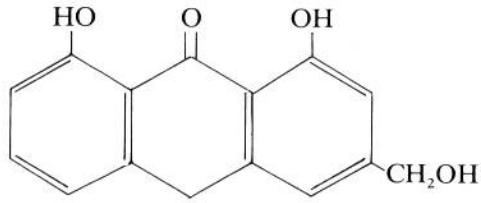
TLC investigation of the fluorescent compound separated from the bandage extracts suggests that it is also umbelliferone (Table 11). A further similarity between the wrapping extract and the galbanum solution is the presence in both of a compound (hRf 32, Table 10) which gives a green colour with the antimony trichloride reagent. Thus it seems possible that the gum resin galbanum which has previously only been reported as an ingredient of Ancient Egyptian toiletries and ointments[12] was used to impregnate the wrappings of this mummy.

The aqueous extracts formed a very high percentage of the total extractive for the lower layers of the bandage but little indication is given by either Lucas[12] or Baumann[2] of water-soluble substances which might have been used in Ancient Egypt, either in mummification and wrapping or in cosmetics and perfumes.

Those suggested include honey, aloes (dried juice of the leaves of *Aloe* spp., Liliaceae), extract of tamarind

fruits (*Tamarindus indica*, Leguminosae) and 'extract of Cassia fruits'. It is not clear from the accounts whether 'Cassia' in this context refers to *Cassia* spp. (Leguminosae) or to *Cinnamonum cassia* (Lauraceae), source of an aromatic bark. Since the former are indigenous to the Middle East and the latter to China, it is probable that *Cassia* spp. is intended.

Honey consists principally of invert sugar and water[23] and is considered to be absent from the bandage extract since fructose was not detected among the sugars in the aqueous extracts by paper chromatography (Table 14). Aloes and *Cassia* spp. both contain anthraquinone glycosides, for example, glycosides of aloe-emodin anthrone (II) found in aloes.



II

Glycosides of this type are detectable by Bornträger's reaction[23] but this gave a negative result for the bandage extract (Table 12). It thus seems unlikely that either honey, aloes or extract of *Cassia* fruit are present in any quantity in the extract. The pulp of tamarind pods is characterized by the presence of free organic acids (approximately 10% tartaric, citric and malic) and their salts (approximately 8% potassium hydrogen tartrate).[23] The aqueous extracts of the bandage were acid to litmus, although the tests for both carboxylic acids and phenols were negative (Table 12). The results of TLC examination for tartaric, citric and malic acids were inconclusive (Table 13): not all the three acids could be detected in aqueous extracts of tamarind in every system used, although in all systems the overall appearance of the chromatograms of the methanol and water extracts of tamarind and those of the bandages was similar.

Galbanum was considered to be a possible constituent of the chloroform and petroleum extracts. Were this to be the case, the gum component would be extracted by water. To check for the presence of gum from galbanum, the aqueous extracts from the bandages and from a sample of galbanum were each hydrolysed and the hydrolysates compared with those of acacia, myrrh and olibanum. The results (Table 14) indicated the presence in the bandage extracts of galactose, arabinose and an unidentified monosaccharide of low hRf, a combination which was found from galbanum but not from acacia (rhamnose present), myrrh or olibanum (unidentified compound giving a bright pink colour with aniline phthalate reagent present in both these extracts). The evidence from PC of the sugars in hydrolysed aqueous extracts of the wrappings thus tends to confirm that galbanum was used to impregnate the bandages.

Gelatin was found as glue on the surface of the outer bandages, therefore it was possible that it had also been used to impregnate the fabric. However, the absence of protein and amino acids from the aqueous extracts (Table 12) shows this not to be the case.

The use of chromatographic analysis has demonstrated that a complex mixture of substances was used to impregnate the bandages and it is likely that in the present study only those have been identified which are:

(a) currently known to have been available to the Ancient Egyptians
(b) used in relatively large quantities
(c) sufficiently chemically stable to have survived storage for 2,000 or more years.

TABLE 12 Spot tests on aqueous extracts of bandages

Test	Results from extract of 1770/			
	1	2	3	4
1. Picric acid (protein)	slight ppt	—	—	—
2. (a) Hydrolysis (HCl) (b) Ninhydrin (amino acids)	—	—	—	—
3. (a) Neutralize by NaOH; (b) Fehlings (free reducing sugars)	+	+	+	+
4. (a) Hydrolysis (HCl) (b) Fehlings (combined reducing sugars)*	+	+	+	+
5. Litmus	H^+	H^+	H^+	H^+
6. (a) ppt in dil. HCl, (b) Sol. in NaOH	+	+	+	+
7. Na_2CO_3 (carboxylic acids)	—	—	—	—
8. $FeCl_3$ soln (phenols)	—	—	—	—
9. Bornträger-test Et_2O extract after hydrolysis (HCl) and oxidation ($FeCl_3$) with dil. NH_4OH (anthraquinone glycosides)			—	

* All +ve results here — subjectively judged that more ppt. formed after hydrolysis than before.

TABLE 13 Results of TLC comparison of aqueous and methanolic extracts of mummy bandage with extracts of tamarind and reference plant acids

hRf	Colour with $NH_4OH/AgNO_3$	Presence (√) or absence (—) in solutions of:						
		Tartaric acid	Malic acid	Citric acid	Tamarind (MeOH)	Tamarind (H_2O)	Bandage (MeOH)	Bandage (H_2O)
System A								
44		√	—	—	√	√	√	√
57		—	—	√	(√)	—	(√)	(√)
61		—	√	—	(√)	—	(√)	(√)
System B								
25		—	—	√	(√)	(√)	(√)	(√)
40		—	√	—	(√)	(√)	(√)	(√)
41		√	—	—	(√)	—	(√)	—
64		—	—	—	√	√	√	√
System C								
14	brown	—	—	√	√	√	(√)	(√)
19	black	√	—	—	√	√	√	(√)
32	pale brown	—	√	—	—	—	—	—
58	grey-white	—	—	—	√	√	√	√

TABLE 14 Results of PC comparison of hydrolysed aqueous extracts of mummy bandage with those of acacia, myrrh, olibanum and galbanum

hRf	Colour with aniline phthalate reagent	Presence (√) or absence (—) in solutions of:										
								hydrolysates of				
		arabinose	dextrose	galactose	rhamnose	xylose	fructose	acacia	myrrh	olibanum	galbanum	aq. ext. of bandage
System A												
9											√	√
14											√	√
21				√							√	√
27		√									√	√
23			√								—	—
25							√				—	—
System B												
5	brown							√	√	√	√	√
12	brown			√				√	√	√	√	√
20	pink	√						√	√	√	√	√
14	brown		√					(—)	(—)	(—)	(—)	(—)
33	brown				√			√	—	—	—	—
23	pink/purple					√		—	—	—	—	—
39	bright pink							—	√	√	—	—
44	grey							—	—	√	—	—
System C												
35	brown							—	√	√	√	√
38	brown		√					—	—	—	—	—
40	brown			√				√	√	√	√	√
45	pink/purple					√		—	—	—	—	—
46	bright pink							—	√	√	—	—
53	pink	√						√	√	√	√	√
61	brown				√			√	—	—	—	—

With these reservations, the conclusions from the present investigation are that the bandages were made of linen cloth impregnated with a mixture containing beeswax, bitumen (except the outer layers), galbanum and a water soluble substance or substances, possibly tamarind extract. It is interesting to speculate as to how the bandages were coated with this mixture of substances containing both water insoluble and water soluble components. Perhaps the most likely explanation is that a molten mixture was prepared and used either for dipping the bandages or brushing on to the surface. That the bandages were not matted together and could be separated easily, indicates that they were fairly dry when applied. The final stage of wrapping seems to have been to use strip bandages which were impregnated with a mixture of substances (which apparently excluded bitumen) and to secure these neatly with glue.

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